COMPARISON OF MEAN PLATELET VOLUME IN TYPE 2 DIABETICS ON INSULIN THERAPY AND ORAL HYPOGLYCAEMIC AGENTS

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BONAFIDE CERTIFICATE

This is to certify that dissertation named **"COMPARISON OF MEAN PLATELET VOLUME IN TYPE 2 DIABETICS ON INSULIN THERAPY AND ORAL HYPOGLYCAEMIC AGENTS"** is a bonafide work performed by Dr.V.Rukmani Prabha, post graduate student, Department of Internal Medicine, Kilpauk Medical College, Chennai-10, under my guidance and supervision in fulfillment of regulations of the Tamilnadu Dr. M.G.R Medical University for the award of M.D. Degree Branch I (General Medicine) during the academic period from May 2012to April 2015.

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DECLARATION

I solemnly declare that this dissertation **"COMPARISON OF MEAN PLATELET VOLUME IN TYPE 2 DIABETICS ON INSULIN THERAPY AND ORAL HYPOGLYCAEMIC AGENTS"** was prepared by me at Government Kilpauk Medical College and Hospital, Chennai, under the guidance and supervision of **Prof.Dr. S. Mayilvahanan M.D.**, Professor, Department of Internal Medicine, Government Royapettah Hospital, Chennai.

This dissertation is submitted to **The Tamil Nadu Dr. M.G.R. Medical University, Chennai** in partial fulfillment of the University regulations for the award of the degree of **M.D. Branch I (General Medicine)**.

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INTRODUCTION

Diabetes mellitus is fast attaining epidemic proportions in India ,with nearly 62 million individuals diagnosed with the disease. Apart from the macrovascular complications they are at risk of developing microvascular complications like retinopathy, nephropathy and neuropathy associated with high morbidity Increased platelet reactivity and insulin resistance are considered to be the prime determinants of all the vascular complications in diabetes . Recent studies point to the role of mean platelet volume to be an important marker for thromboembolism.

AIMS AND OBJECTIVES

- To determine the association between mean platelet volume and type 2 diabetes mellitus

- To compare the values of Mean Platelet Volume in type 2 diabetic subjects on insulin therapy and those on oral hypoglycaemic agents.

- To determine the association of microvascular complications with mean platelet volume.

MATERIALS AND METHODS

After obtaining an informed consent from the patients a detailed clinical history will be obtained including the duration since diagnosis of DM and

commencement of treatment, followed by a physical examination including fundus examination.Then lab investigations like fasting blood glucose,
2 hr postprandial blood glucose,Hb A1C, complete haemogram, mean platelet volume will be performed and results will be correlated

RESULTS

Among the 50 subjects selected for the study, 19 were on insulin therapy and 31 were on oral hypoglycaemic agents.the MPV was found to have a positive correlation between fasting blood glucose , postprandial blood glucose , HbA1C , and presence of retinopathy. The mean platelet volume was significantly lower in the group on insulin therapy than those taking OHAs, and this observation was statistically significant.(p value =0.000)

CONCLUSION

The Mean platelet volume was found to be lower in the insulin therapy group indicating that the early initiation of insulin therapy in the diabetic population not only controls blood sugar but also lowers the MPV and protects from the vascular complications. There was a definite positive correlation between microvascular complications and MPV indicating a increased platelet reactivity among these individuals. Thus MPV can serve as a cost effective tool in monitoring the platelet reactivity and monitoring vascular complications of diabetes.

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INTRODUCTION

Diabetes mellitus is fast attaining epidemic proportions in India ,with nearly 62 million individuals diagnosed with the disease.[1]India became the forerunner of the global diabetic population in the year 2000 with 31.7 million diabetic people , closely followed by China with 20.8 million and United states with 17.7 million.[1]Recent estimates published by Wild et al predicted a doubling of the global diabetic population from 171 million in 2000 to 366 million in 2030 with a highest increase in our nation.[1]

The macrovascular complications of diabetes include cardiovascular, cerebrovascular and peripheral arterial disease. Hyperglycemia as such or diabetes mellitus or both is said to account for nearly 3 million deaths due to coronary events every year. 60 % of the deaths in diabetic people is due to cardiovascular diseases. Adverse coronary events occur at a much younger age in diabetic individuals and the incidence is almost the same in both men and women.

Among the 239 diabetic patients followed in the Framingham Heart Study of 1948 , there was a three fold increase in cardiovascular mortality, leading to diabetes being considered a risk equivalent of CAD[3]. In the GUSTO-1 trial the analysis of patients with ST elevation MI showed an increase in 30 day mortality in the diabetic population compared to non diabetics. .

Similarly the odds of a diabetic person developing ischaemic stroke was 2-3 times higher than the non diabetic individual.[4-7]

Apart from the macrovascular complications they are at risk of developing microvascular complications like retinopathy, nephropathy and neuropathy associated with high morbidity[8]. Numerous mechanisms could be sought to explain this increased risk, however the most important among all would be the presence of a pro inflammatory and pro thrombotic state. And the prominent role played by platelets cannot be undermined. Increased platelet reactivity and insulin resistance are considered to be the prime determinants of all the vascular complications in diabetes. This is re iterated by the outcome of the TRITON –TIMI 38 study which showed a significant decrease in ischaemic events with aggressive antiplatelet therapy in the diabetic population in comparison to anti diabetic people.

Platelets have a vital role to play in maintaining hemostasis. The measurement of platelet size and volume are considered as important determinants of platelet function. Recent studies point to the role of mean platelet volume to be an important marker for thromboembolism[9], MI and ischaemic stroke. Platelets when larger in size are considered to be more reactive with increased pro-thrombotic factor thromboxane A2 release and hence a increased thrombogenic potential[10] . The mean platelet volume was found to be higher in diabetic population than the non diabetics and it was found

to improve with good glycemic control(11). MPV is also considered to influence the development of micro and macro vascular complications of diabetes mellitus which has been dealt in this study.

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LITERATURE REVIEW

DIABETES MELLITUS AN OVERVIEW

Diabetes mellitus is characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both.

THE INDIAN SCENARIO

Diabetes mellitus in India is a constantly rising problem. Recent studies indicate that by 2030 there will be up to 79.4 million individuals suffering from diabetes mellitus in India.[1]

The morbidity and mortality produced by diabetes and its complications are so varied and widespread that they will be a substantial burden on the family as well as the society. The fact that there is increasingly numerous data supporting the occurrence of complications at much younger ages in India. The current trend of migration of the population from the rural to the urban areas, changes in life style favouring sedentary habits and the economic boom have all paved to the increased incidence of diabetes in our country. Inspite of this rapid expansion in the diabetic population there remains a definite void in relation to the studies on DM , bringing out the cultural, socio economic ,geographical influence on the disease.

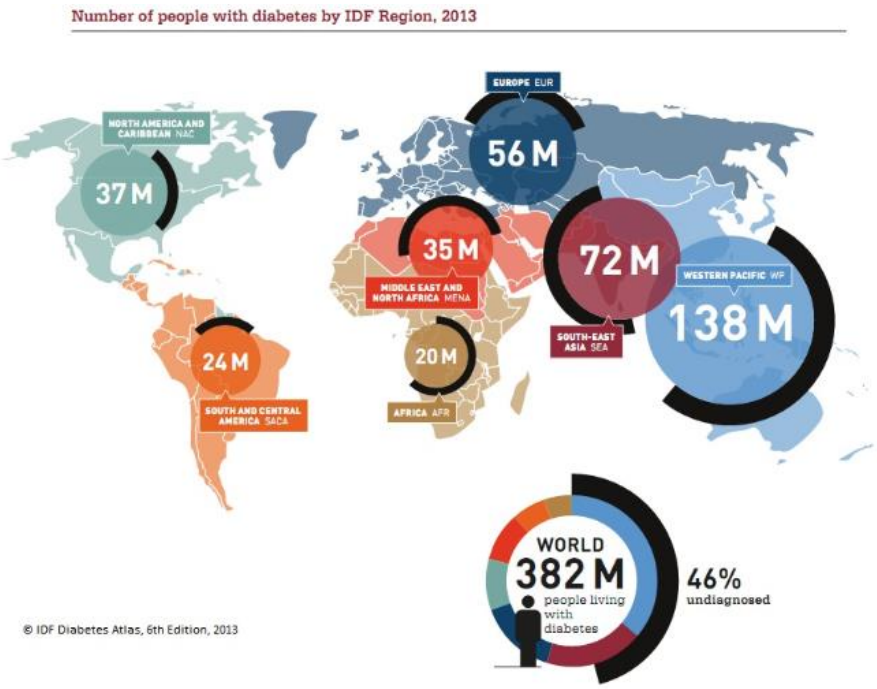


Figure 1 PREVALANCE OF DIABETES 2013,IDF

Diabetes in Indians is found to occur at a relatively lower body mass index compared to the European or other counterparts.[12,13] thus Indians who are non-obese and with a lower BMI are at equal risk of developing diabetes. Also Indians are predisposed genetically to develop coronary artery disease as a result of increased incidence of dyslipidaemia and low HDL. All these factors make us Indians more susceptible for the development of diabetic complications at a much lower age (20-40 years) in comparison with the Caucasians (>50 years).[14] Thus the initiation of early screening programs to detect individuals with pre diabetes in high risk population like children and

adults with BMI ≥25 and pregnant women, can pave way to regulate and yield positive health outcomes .

The presence chronic state of hyperglycemia in diabetes leads to prolonged damage, impaired function, and failure of various organ systems, mainly the kidneys, eyes, kidneys, the nervous system , the heart, and blood vessels.

Various mechanisms are attributed to the development of diabetes. Some of them being an autoimmune destruction of the beta-cells of the islet of pancreas with resultant deficiency of insulin and abnormal metabolism leading to resistance to the action of insulin. The common factor leading to defects in the metabolism of carbohydrate, fat, and proteins is the defective action on target tissues produced by a deficiency of insulin.

The classical triad of symptoms associated with hyperglycemia includes polyuria, polydipsia, weight loss, and on occasions associated with polyphagia, and blurring of vision along with an increased susceptibility to infections. The acute often life-threatening complications of hyperglycemia and diabetes are ketoacidosis or Non -ketotic hyperosmolar coma. The long-term complications of diabetes include retinopathy, nephropathy and neuropathy. With the risk of developing potential loss of vision , chronic renal

failure; occurrence of ulcers in foot , rarely amputations, and Charcot joints; also the resulting autonomic neuropathy can lead to GI, cardiovascular ,genitourinary and sexual dysfunction. The macrovascular complications are more common in diabetes and they are highly prone to atherosclerotic cardiovascular, cerebrovascular and peripheral arterial, diseases.

CLASSIFICATION

Diabetes is mainly classified into four major categories as

- Type 1 diabetes mellitus
- Type 2 diabetes mellitus
- Other specific types
- Gestational diabetes mellitus

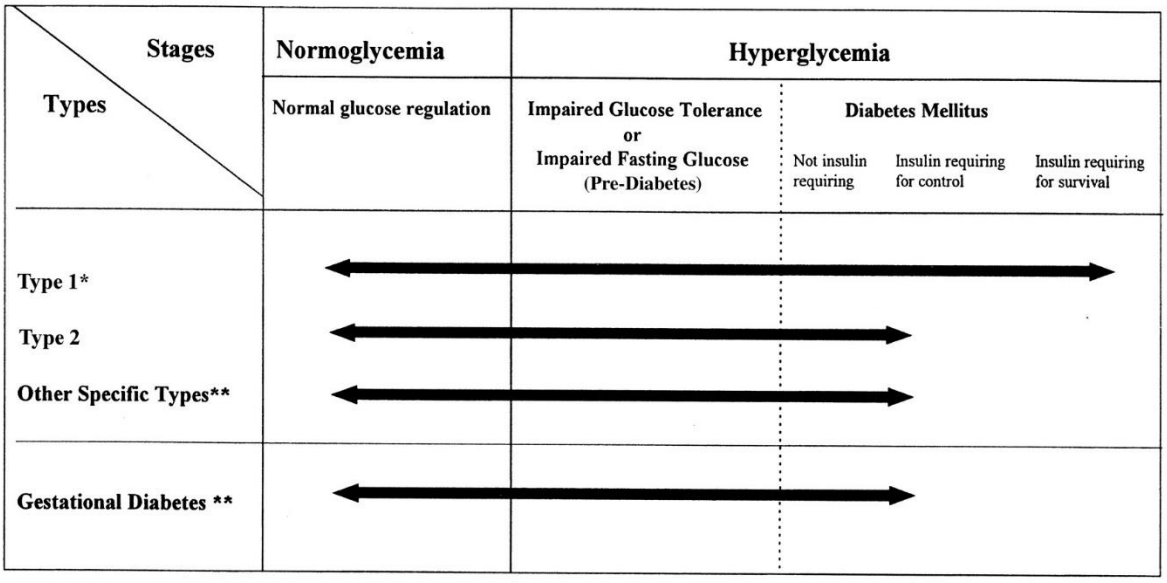


Figure 2 natural history of DM

Type 1 diabetes

Its associated with the destruction of beta cells leading to absolute deficiency of insulin, often called as the immune mediated diabetes. Only 5–10% of the overall diabetic population fall under this type. Earlier referred as insulin dependent diabetes or juvenile-onset diabetes. The presence of auto antibodies against the islet cell , insulin, GAD (GAD65), and to the tyrosine phosphatases indicate an immune mediated insult[15]. 85–90% of patients with type 1DM have one or more of these antibodies. Further, there exists a strong HLA associations in this form of diabetes. Early adolescence or childhood is the most common age group for the occurrence of this immune mediated pathology, however it can occur at any age as well as late as in the 8th and 9th decades. There is an increased incidence of autoimmune diseases in these subjects.

Idiopathic diabetes.

Some persons with type 1 DM have no specific etiology, but exhibit insulin deficiency and there is a clear absence of any of the auto antibodies ,but have no evidence of autoimmunity, and they are denoted as persons with idiopathic diabetes for the lack of a clear etiology.[16]

Type 2 diabetes

The underlying process can be explained by a resistance to insulin action with a relative deficiency of insulin or a clear defect in the secretion of insulin associated with a IR . 90–95% of diabetics fall under this category. It's also called as non–insulin-dependent diabetes, or adult onset diabetes.

A significant proportion of patients with type 2 DM are obese and this can contribute to insulin resistance as discussed further in this study. And those who are not obese are found to have a higher incidence of visceral adiposity. This form can go undiagnosed for a prolonged period as the onset of hyperglycemia takes several years and the earlier stages are not usually characterised by the typical symptoms.

CATEGORIES OF INCREASED RISK FOR DIABETES

The Expert Committee on Diagnosis and Classification of Diabetes Mellitus [17] identified a group of people who did not fit the diagnostic criteria for diabetes yet had glucose values higher than what was considered normal. And they were classified as having impaired glucose tolerance. They were referred to as having pre-diabetes .With an increased tendency to develop diabetes in future. Lifestyle modifications , increase in the physical activity and

a weight loss of 5–10% , and Specific drugs have been proven to prevent or delay the development of diabetes in people with IGT.

Categories of increased risk for diabetes*

FPG 100 mg/dl (5.6 mmol/l) to 125 mg/dl (6.9 mmol/l) [IFG]
2-h PG in the 75-g OGTT 140 mg/dl (7.8 mmol/l) to 199 mg/dl (11.0 mmol/l) [IGT]
A1C 5.7–6.4%

Figure 3 impaired glucose tolerance/pre diabetes

Blood Test Levels for Diagnosis of Diabetes and Prediabetes

	A1C (percent)	Fasting Plasma Glucose (mg/dL)	Oral Glucose Tolerance Test (mg/dL)
Diabetes	6.5 or above	126 or above	200 or above
Prediabetes	5.7 to 6.4	100 to 125	140 to 199
Normal	About 5	99 or below	139 or below

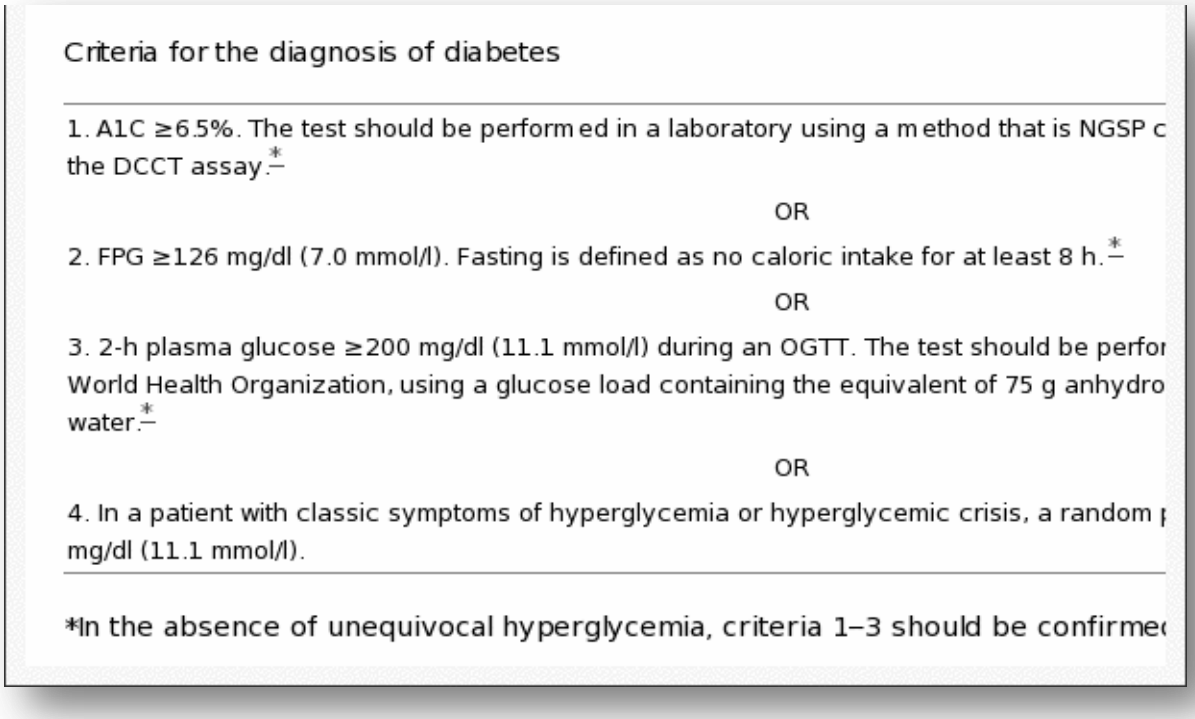
Definitions: mg = milligram, dL = deciliter
For all three tests, within the prediabetes range, the higher the test result, the greater the risk of diabetes.

Figure 4 diagnosis of dm and pre diabetes

Criteria for diagnosis of type 2 DM:

This takes into account fasting blood glucose and a 2 hr plasma glucose on OGTT, and HbA1c which is a marker of chronic glycemic status over a period of 2- 3 months.

DIAGNOSTIC CRITERIA FOR DIABETES MELLITUS



Criteria for the diagnosis of diabetes

1. A1C ≥6.5%. The test should be performed in a laboratory using a method that is NGSP c the DCCT assay.*

OR

2. FPG ≥126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.*

OR

3. 2-h plasma glucose ≥200 mg/dl (11.1 mmol/l) during an OGTT. The test should be perfor World Health Organization, using a glucose load containing the equivalent of 75 g anhydro water.*

OR

4. In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random p mg/dl (11.1 mmol/l).

*In the absence of unequivocal hyperglycemia, criteria 1–3 should be confirme

Figure 5 diagnostic criteria for DM

INSULIN RESISTANCE AND THE PATHOGENESIS OF TYPE 2 DIABETES

Insulin resistance indicates the to resistance to its various metabolic effects , like the suppression of endogenous glucose production, the stimulatory effects on the peripheral (mainly skeletal muscle) glucose uptake and synthesis of glycogen, and finally the inhibition of adipose tissue lipolysis.

Numerous studies have demonstrated the occurrence of insulin resistance prior to the onset of type 2 diabetes by nearly 10 to 20 years. Insulin resistance is considered as the best clinical predictor of subsequent development of type 2 diabetes[18,19].. However, for the occurrence of overt diabetes mellitus there is a necessity for an additional impairment in insulin secretion[18] .When the β-cell function adequately , individuals continue to compensate indefinitely for insulin resistance by developing hyperinsulinemia . But however , the risk of developing atherosclerosis is found to be similar in non-diabetic individuals with insulin resistance patients with overt with type 2 diabetes [20].

Contributors of tissue specific IR:

SKELETAL MUSCLE

A universally recognized feature of type 2 diabetes is resistance to insulin occurring in the skeletal muscle , which could be either due to genetic factors or metabolic factors. Skeletal muscle is quantitatively the most prominent organ involved in the homeostasis of glucose, as it accounts for ~80% of glucose utilization following glucose ingestion or infusion. Thus, the reduced capacity of insulin to stimulate the disposal of glucose by skeletal muscle is of prime importance to whole-body glucose homeostasis. Impairment of glucose uptake and its phosphorylation which are dependent on insulin have been identified at initial stages of type 2 diabetes[21]. The experimental studies have showed skeletal muscle playing a vital role as a site of insulin action, still there is indication of presence of alternate pathways leading to glucose uptake and translocation of GLUT4 in skeletal muscle which is found to for the lack of insulin receptor.

The transport of 3- *O* -methylglucose into the skeletal muscle from patients with type 2 diabetes which is dependent on insulin is found to be substantially lower than in normal controls, demonstrating that decreased skeletal muscle uptake contributes to impaired peripheral glucose uptake[22,23]. A uniform finding in both obesity and type 2 diabetes is decreased insulin receptor substrate-1 (IRS-1) associated tyrosine phosphorylation and Phosphotidyl ionositol 3-kinase activity in skeletal muscle[24,25]. In addition to this down regulation of proximal insulin signaling, several negative regulators of insulin

signaling are up regulated in insulin resistance. Plasma-cell differentiation factor-1 (PC-1) is a membrane glycoprotein that acts as an intrinsic inhibitor of insulin receptor tyrosine kinase activity[26] It has been suggested that increased expression of PC-1 in skeletal muscle of obese subjects is more strongly associated with down regulation of the insulin receptor tyrosine phosphorylation , than with decreased insulin receptor expression[27] .Similarly there is an increase in the activity of membrane bound tyrosine phosphatase especially that of PTP-1B[28], which negatively regulates phosphorylation of insulin receptors, leading to impaired peripheral uptake.

ADIPOSE TISSUE

Insulin resistance in adipose tissue is characterized by a reduction in the suppression of lipolysis in the adipose tissue leading to an elevated level of free fatty acids. This is seen in both obese insulin-resistant individuals and patients with type 2 diabetes .The occurrence of the same defect in first-degree relatives of patients who have normal glucose tolerance [29] indicates that abnormal suppression of plasma free fatty acids mediated through insulin is early abnormality in people with a genetic predisposition to IR. The reduction or absence of aP2, a fatty acid binding protein found in adipose tissue results in reduction in adipose tissue lipolysis and an elevated mass of adipose tissue[30] .this is accompanied by a paradoxical decrease in plasma lipids and a

better insulin sensitivity and better secretion of insulin [31]. This brings to light that increase of adipose tissue mass may have beneficial effects against insulin resistance. This strategy of adipose tissue serving a protective function to other tissues from the deleterious effects of excessive FFA is reiterated by the fact that overexpression of GLUT4 in adipose tissue of experimental models produces both an improvement in sensitivity to insulin and increase in mass of adipose tissue.

LIVER

The concept of glucose production by liver is an area of controversy, as to if it is regulated by insulin or is being regulated by the extra hepatic metabolic effects. The proposed mechanism is that, the production of glucose is being suppressed by decreasing the input of free fatty acids and amino acids from adipose tissues and muscle, this being mediated by insulin is found to reduce hepatic gluconeogenesis [32]. For this process to occur normally there has to be an adequate response to insulin at the level of adipose tissues.it is this ability of insulin in the periphery to control the glucose production that is responsible for supressing circulation of free fatty acids.

The onset of overt diabetes requires the presence of insulin resistance at the hepatic level. Studies indicate that hepatic insulin resistance is

due to an inherent abnormality of insulin signaling occurring at the hepatocyte.

BRAIN

Insulin exerts a central action on the hypothalamus, is is responsible for suppression of appetite (centrally mediated) and various other metabolic effects. Resistance to this action of insulin in the hypothalamus can result in centrally mediated insulin resistance. There is a wide distribution of insulin receptors in several areas of brain[34] which are associated with the regulation of satiety [34].the main pathway of glucose disposal in most of neurons is through an insulin-independent mechanism , however in the hypothalamus and other specific areas of the brain, its mediated by the insulin-responsive glucose transporter GLUT4.

This proposed mechanism of central inhibition mediated by insulin also greatly suppressed ability of exogenously infused insulin to bring down the hepatic glucose output. This clearly points to the vital role played by the insulin receptor in the hypothalamus in regulating the of glucose metabolism of liver.

GENETIC CAUSES OF INSULIN RESISTANCE

There is a defect in both the action and secretion of insulin in type 2 diabetes, and both are considered to be genetically predetermined. Furthermore, there is a nearly 100% concordance in diagnosis of type 2 diabetes

between monozygotic twins but only a 20% concordance between dizygotic twins [88].

INSULIN RECEPTOR MUTATIONS

There are nearly 100 mutations occuring naturally in the insulin receptor gene and most of them are associated with resistance to insulin action.[36]IR also occurs with the complete absence of these receptors [37]. Leprechaunism is the most severe form of such mutation in insulin receptor syndromes, it's characterized by IUGR and characteristic dysmorphic facies comprising of thick lips, low set ears, prominent eyes, upturned nostrils, and thick skin with the absence of subcutaneous fat. The milder form of such mutation is the Rabson- syndrome where patients have life expectancies nearly 15 years. These syndromes being characterised fasting hypoglycaemia and prominent mitogenic features, inspite of a significant resistance to the actions of insulin, indicate that these mitogenic effects are mediated by the high circulating insulin levels exerting its action through the homologous IGF-I receptor.

MUTATIONS IN PPAR GAMMA

The nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) appears to play a vital role in both adipocyte differentiation and

insulin action, thus denoting the important interactions between these two phenomena .The mutation associated with this (dominant-negative and other types of mutations) result in the features of severe insulin resistance .these syndromes are characterised by partial lipodystrophy involving the limbs and buttocks, with specific sparing of the adipose deposits of the abdomen and likewise sparing the face[38].This characteristic lipodystrophy is explained by defect of the subcutaneous adipose tissue in the abdomen to trap and store the free fatty acids in postprandial state . A Pro12Ala polymorphism in the PPARγ gene is found to be associated with better sensitivity to insulin .In general, the expression of PPARγ is high in the adipose tissue of obese individuals ,however this is seen to be down regulated in individuals with a low-calorie diet [39].

IMPACT OF OBESITY ON INSULIN ACTION

The proportion of body fat in mammals is spread across a wide range , from 2% to 50% of total body mass .Obesity or excess of body fat, plays a significant role in the pathogenesis of IR and greatly elevates the risk of developing type 2 diabetes .Drastic hike in the intake of low-cost fat and simple carbohydrate calories have lead to the massive increases in occurrence of of overweight and obesity.

Adipose tissue acts as the body's main site for storage of energy , and it regulates the insulin action on the entire body mainly through the release

of free fatty acids and by the production of proteins derived from adipose tissue. These adipose derived proteins include the pro inflammatory peptides, and newer hormones which exerts a significant effect on insulin action and the metabolism of glucose.

ADIPONECTIN :

It's a secretory protein that increases the sensitivity to insulin [40]. Decreased levels of these hormones in circulation is seen to occur in conjunction with IR and obesity in humans and in experimental animals. [41] Studies revealed a negative correlation between adiponectin levels and hyperinsulinemia also with the extent of insulin resistance .Chronic restriction of calories (which is said to improve insulin action) results in an increase in levels of adiponectin .Furthermore, higher levels of plasma adiponectin are associated with a substantially decreased risk of development of type 2 diabetes.

Additionally, suppression of the production of glucose in hepatocytes by the sub physiological levels of the hormone insulin is mediated by adiponectin. The ratio of the two oligomeric forms (HMW/ LMW) has recently been shown to correlate more clearly with action of insulin than the total amounts of the hormone in circulation. Pharmacologic activation of PPARγ with thiazolidinediones increases adiponectin levels, the most striking effect is a selective proportional increase in the HMW form with close association with improved hepatic insulin action.

LEPTIN :

Leptin is produced by a defect in the obesity gene and is produced from adipose tissue. The levels of circulating amounts of leptin correlates more clearly with insulin levels in fasting and proportion of body fat. Thus it's appropriate to identify leptin as a marker of obesity and the syndrome of insulin resistance . Acute leptin administration produces an enhanced inhibition of glucose production in liver by insulin without affecting its peripheral action .[42]

However chronic leptin administration induces metabolically favourable changes in body composition that include decreased visceral adiposity and reduced muscle accumulation of triglyceride [43]. These changes in body composition induced by leptin are associated with an enhanced sensitivity to the various metabolic changes in skeletal muscle attributable to insulin.

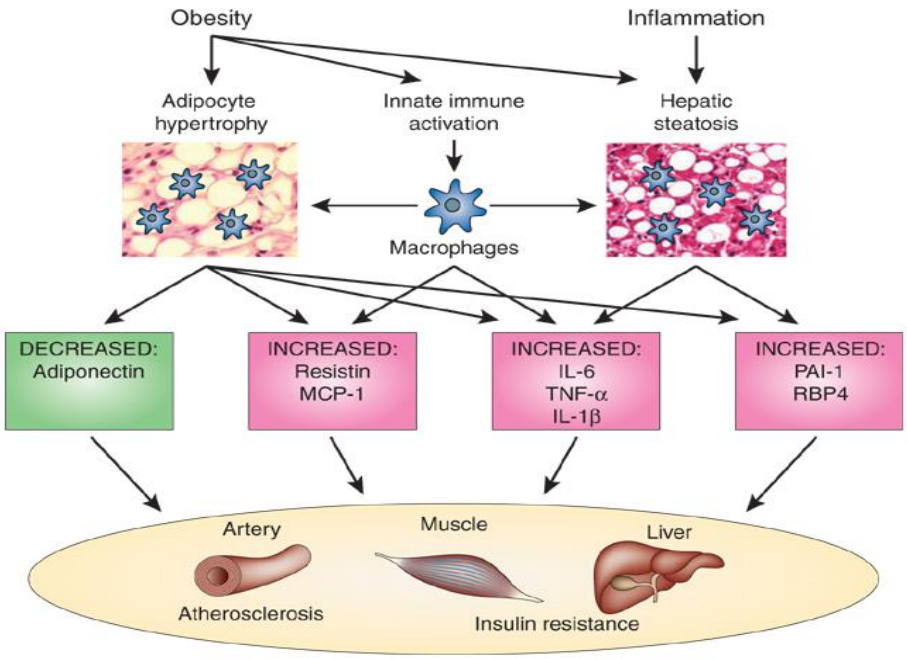


Figure 6 factors contributing to insulin resistance

These favourable effects on body composition and lipid storage may be due to leptin-induced stimulation of β-oxidation via activation of AMPK in muscle and liver. Profound leptin deficiency in is accompanied by impairment of T-cell immunity. Leptin influences the regulation of hormones in the HPO axis and affects the anterior pituitary hormones like prolactin, growth hormone, prolactin, and others as well.

Leptin has anorectic effects which is mediated centrally and resistance to this mechanism occurs in the presence of nutrient excess [(44)]. However, leptin was effective in increasing energy expenditure in obese

individuals after a sustained weight reduction of ~10% of their previous body weight.

RESISTIN

Its a adipose tissue-specific hormone recently and is up regulated during adipogenesis and down regulated by treatment with a PPARγ agonist . The IR produced by infusion of resistin was completely accounted for by a hike in the glucose production rate, suggesting that resistin has rapid inhibitory effects on hepatic rather than peripheral insulin sensitivity. Furthermore, few studies indicate an association between levels of resistin and BMI or with insulin sensitivity among humans [45], however no evidence exists to reveal such a connection.

OBESITY, INFLAMMATION AND IR

There is mounting evidence indicating a more causal relationship between inflammation and insulin resistance and not a mere correlative association. Epidemiologic data from several large studies have demonstrated an association between IR and systemic inflammation in both diabetics (type 2 DM) and the non-diabetic populations [46]. Adipose tissue produces many pro inflammatory molecules, including TNF-α, IL-6, TGF-β, C-reactive protein,

MCP-1, and circulating levels of these “adipokines” are increased in obesity. Pro inflammatory chemicals derived from adipose tissue are found to produce systemic IR and also leading to the pathogenesis of various metabolic complications of obesity, including type 2 diabetes and atherosclerosis .

TNF-α reduces the sensitivity to insulin and enhances the adipocyte lipolysis [47]. Expression of TNF-α is increased in white adipose tissue in obese as well as insulin-resistant states. TNF-α activates NF-κB. Stimulation of production of various other cytokines is the main action of TNF –α. And these cytokines exert their action directly on the muscle.

Lipolysis is found to be increased by IL-6 and has been found to increase values of triglycerides and serum free fatty acids in obesity . Further IL-6 produces insulin resistance at the cellular level in hepatocytes [48]. Current studies indicate an impairment in insulin sensitivity produced by the chemokine MCP-1. Aspirin treatment inhibits the phosphorylation of serine in of IRS-1 of the tumour necrosis factor-α treated cells. This is done by action upon the multiple serine kinases ,thus imparting a beneficial role.

Obesity is associated with increased infiltration of macrophages into adipose tissue. With the onset of obesity, pre-adipocytes are stimulated to secrete the chemokine MCP-1, this is stimulated by secretion of very low amounts of TNF-α.MCP 1 is a chemoattractant specific for monocytes and macrophages [49].Adipocytes produce leptin in higher amounts ,which produces

the transport of macrophages leading to their accumulation in adipose tissue. [50] It also promotes the adhesion of macrophages to endothelial cells. Adipocytes also produce colony-stimulating factor-1 (CSF-1). This CSF-1 is the primary moderator of macrophage differentiation and survival. Once the macrophages are activated they are present in adequate quantity in the adipose tissue. They act in conjunction with the adipocytes and other formed elements to perpetuate a vicious circle. This vicious cycle comprises of recruitment of macrophages and secretion of inflammatory mediators, ultimately resulting i systemic insulin resistance.

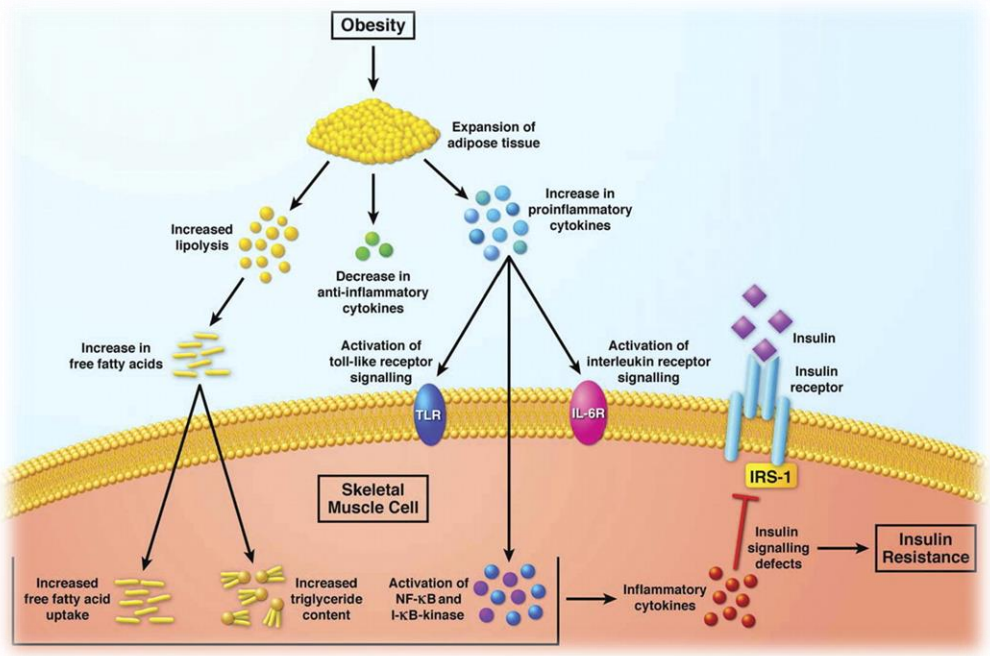


Figure 7 obesity and insulin resisitance pathways

The size of the adipocyte cell is found to correlate significantly with dyslipedemia, markers of IR and risk for developing type 2 diabetes . The amount of visceral fat mass, which takes into account both omental , mesentric and other intra-abdominal fat depots, is significantly correlated with the pathologies associated to obesity than is overall body adiposity . Proposed mechanisms for the negative metabolic effects of visceral fat include unrestrained lipolysis with increased circulating FFA levels, a specific increase in portal delivery of FFA, and unfavourable secretion profiles of adipose-derived proteins. .catecholamines exert a stronger influence on the visceral fat cells by producing lipolysis, than the antilipolysis produced by insulin. This is the inverse of the process occurring in subcutaneous fat.[51]

Furthermore, the portal vein receives the direct venous effluent from the visceral adipose tissue, leading to a high flux of free fatty acids into liver in centrally obese individuals. Increased hepatic delivery of fatty acids results in hepatic insulin resistance and in decreased insulin clearance, which leads to occurrence peripheral insulin resistance. This indicates the presence of a “cross-talk” between adipose depots. It also reiterates the fact that the secretory mediators produced by visceral fat play a significant role in metabolism of subcutaneous fat and in the onset of insulin resistance.

INTRAMYOCELLULAR TRIGLYCERIDE ACCUMULATION

Accumulation of triglyceride intramuscularly leads to the development of resistance to insulin action in the muscle irrespective of the person being obese or lean , or diabetic or non diabetic. In insulin-resistant persons who are obese, there has been a demonstrable impairment in the uptake and metabolisation of free fatty acids . This can be explained by the defect in carnitine palmitoyltransferase-1 (CPT-1) or the steps occurring further downstream in the fatty acid oxidation pathway.

The rate of muscle fatty acid oxidation is influenced by the levels of plasma insulin, delivery of free fatty acid and glucose .[52] this is indicated by the tissue aggregation of of malonyl-CoA. The levels of free fatty acids in circulation is increased in insulin-resistant individuals, also a concurrent defect in the metabolism of FFA occurring at the extramuscular level also contributes to insulin resistance. Thus the higher amounts of FFA delivered to the muscle along with an impairment in the muscle fatty acid oxidation have additive effects on the intramuscular triglyceride accumulation leading to the occurrence of IR in muscle in individuals with obesity and IR.

INTRAHEPATO CELLULAR TRIGLYCERIDE ACCUMULATION

Nonalcoholic hepatic steatosis (infiltration of fat in the liver) is a common observation in people with obesity. This may fall into any of the following stages of steatosis, steatohepatitis, fibrosis, and cirrhosis . Lean , non -diabetic, non-alcoholic subjects who had fatty liver in liver biopsy had a impairment in the insulin -mediated depression of gluconeogenesis and reduction in glucose disposal which is insulin-stimulated (by ~ 50%) and insulin resistance comparable to that of individuals with type 2 diabetes [53].

Hepatic enhancement of FA oxidation was found leading to increased uptake of glucose in the peripheral tissues(insulin-stimulated) even when associated with intra cellular triglyceride accumulation in the muscles. It has been considered that an unidentified factors secreted by the liver increases glucose uptake into skeletal muscle.

ENVIRONMENTAL FACTORS RESULTING IN IR

Medical conditions:

One of the first medical causes of insulin resistance to be identified was the presence of antibodies to insulin receptor (type B insulin resistance) IR is a component of chronic renal failure and uremia , this insulin resistance is however found to improve with dialysis. The cause of the insulin resistance in renal failure is multifactorial. The reduction in muscle mass , impaired physical activity, increased levels of toxins of uremia, hormonal imbalances (elevated

growth hormone and glucagon), altered lipid ratios and metabolic acidosis are considered to be possible mechanisms.

Cirrhosis of liver is commonly associated with abnormal glucose tolerance and insulin resistance. Elevation in circulating FFAs and insulin levels, both are proved to inhibit insulin action.

Insulin resistance is a commonly recognized problem in patients with several types of cancer, particularly malignancies of the gastrointestinal tract and pancreas . It has been proposed that inflammatory mediators contribute to this insulin resistance, particularly TNF-α and IL-6.

Hormonal mediators:

Insulin resistance has been documented in the presence of many syndromes of abnormal hormone levels, particularly states of hormone excess. The following hormones (epinephrine, norepinephrine, cortisol , glucagon, and growth hormone) play vital roles in opposing the insulin action after hypoglycaemia. In Cushings syndrome hyperactivity of the hypothalamic-pituitary-adrenal axis and that the enhanced cortisol secretion contributes to insulin resistance.

Increased levels of norepinephrine or epinephrine or both contribute to insulin resistance in phaechromocytoma. Growth hormone acts at multiple states to inhibit insulin actions. This includes suppression of the insulin receptor

phosphorylation and its main signalling molecule, the IRS-1, leading to the development of insulin resistance in acromegaly.

SYSTEMIC EFFECTS OF NUTRIENT EXCESS

Glucose toxicity refers to the inhibitory effects of chronic hyperglycemia on insulin secretion and action . Hyperglycemia induced insulin resistance includes- down regulation of the glucose transport system by hyperglycemia and a defect in insulin-stimulated glycogen synthesis. The desensitization of the glucose transport system induced by glucose could be prevented by glutamine analogues that inhibit glutamine : fructose-6-phosphate amidotransferase (GFAT)[54], the initial and rate-limiting enzyme in hexosamine biosynthesis .Protein kinase C is activated by diacylglycerol (a component of the intracellular component), the increase in concentration of diacyl glycerol is found be dependent on glucose. The muscles when exposed to hyperinsulinemia PKC inhibitors blocked insulin desensitization by glucose.

As discussed earlier insulin resistance is commonly associated with inappropriate suppression of oxidation of fatty acid . Similarly there exists a definite inverse correlation between the oxidation of glucose and lipids diabetic subjects who are obese. Elevated amounts of free fatty acids in plasma results in the down regulation of insulin signalling occurring at the level of human skeletal muscles. Increase in the free fatty acid levels can also perpetuate

inflammation. The high levels of fatty acids in plasma also lead to defective suppression of hepatic gluconeogenesis.

OXIDATIVE STRESS AND AGING

Activation of common stress-activated signalling pathways such as nuclear factor-KB, MAPK, and NH2-terminal JKinases ,stress-activated protein kinases([56] by glucose and possibly free fatty acids leads to an impairment in insulin sensitivity and secretion.

The final "environmental" factor that contributes to insulin resistance is increasing age. The multiple factors associated with aging leading on to IR are higher fat mass especially the increase in visceral adiposity , elevated levels of inflammatory proteins in circulation, and an increase in accumulation accumulation of triglycerides within the cells[57]. A consistent documentation about aging is that restriction of calories in a chronic fashion markedly improves survival and protects from developing IR[58]. Recent studies point that the reduction in mitochondrial functions associated with advancing age like that of oxidative phosphorylation , contributes to development of insulin resistance in the senior population.

As reviewed above, insulin resistance occurs as a result of the complex interactions of genes, obesity, and "environment," with the latter

including nutritional and hormonal factors, as well as advancing age. And this insulin resistance plays a pivotal role in developing type 2 DM , atherosclerosis and the platelet dysfunction underlying all these conditions.

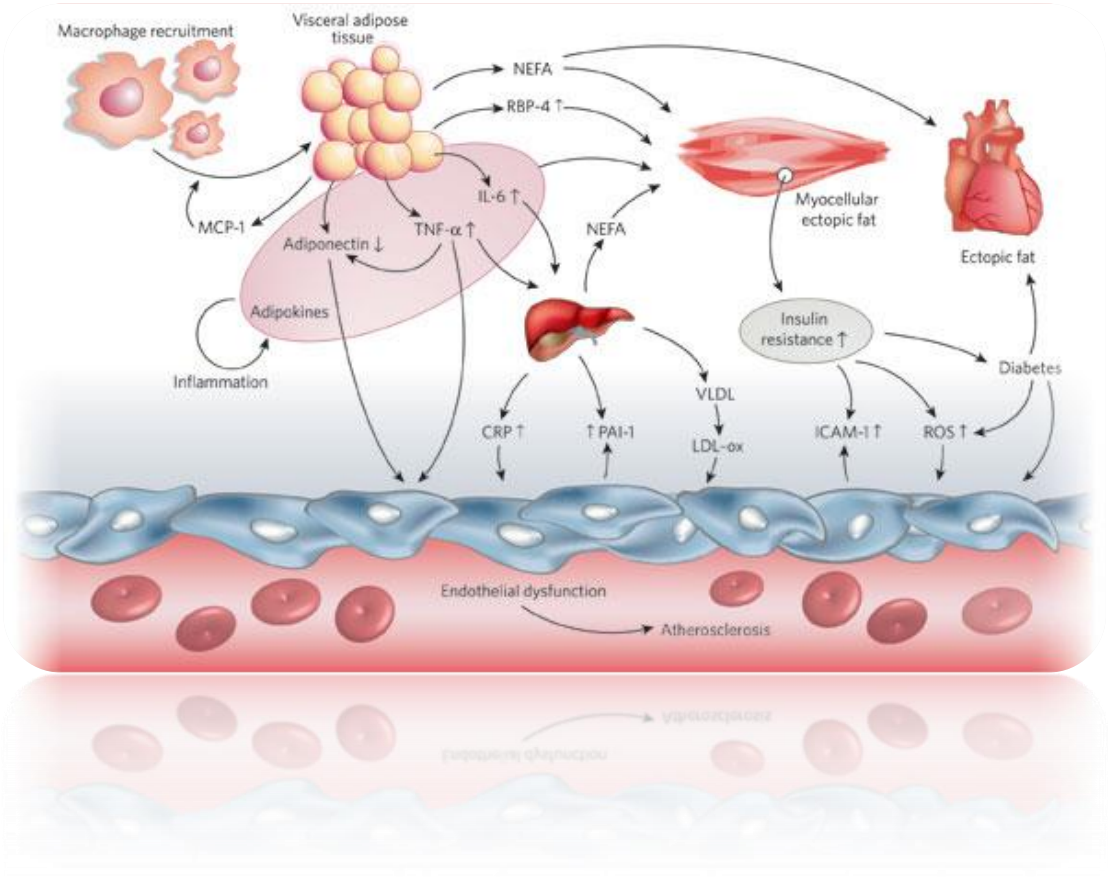


Figure 8 inflammatory mediators in insulin resistance

PATHOLOGY OF MICROVASCULAR COMPLICATIONS

The classical triad of microvascular complications include retinopathy, nephropathy and neuropathy. Recent studies indicate that the pathological mechanism behind these complications is not entirely a

microvascular insult but other factors also play a role. The occurrence of cellular dysfunction in the nonvascular tissues in the early stages of DM reiterate this fact. The question as to why only certain tissues in particular are affected has to be answered.

THE CHANGES IN VASCULATURE

The vascular cells undergo growth and apoptosis preferentially in diabetes. There is a preferential activation of the apoptotic pathways in the pericytes of retinal microvasculature , in the renal capillaries and the micro capillaries of heart .While there is activation and proliferation of microvessels in proliferative retinopathy, and atherosclerotic lesions. The chemical mediators of the apoptotic pathways are hydrogen peroxide, NF κ B, JNK1[59].

The thickening of basement membrane forms the main pathological feature of microangiopathy , and this is a universal phenomenon occurring in both the vascular and nonvascular structures. The basement membranes of the capillaries in retina normally thickens with age , this however occurs at an accelerated phase in diabetic population. In renal pathology, there is an expansion of the extracellular matrix which is seen as a thickened GBM, mesangial expansion and tubulointestitial fibrosis.[60] An increase in synthesis and reduced degradation of the extracellular matrix proteins is seen as the underlying defect. Diabetics display an overexpression of collagen type 4 and

fibronectin. Endothelial dysfunction is a very important pathological process in microvascular changes of diabetes and it is dealt in detail in subsequent chapters.

TISSUE SPECIFIC VASCULAR CHANGES

The changes in retinal microvessels include basement membrane thickening , increase in the vessel permiability, destruction of pericytes and all these leading to microaneurysm formation. These changes lead to occlusion of the vessels, reduction in retinal blood flow, angiogenesis, hemorrhage and finally retinal detachment. The normal ratio of pericytes to endothelial cells in retinal capillaries is 1:1[60] , in diabetics there is steep drop and the ratio falls to 1:10 in moderate NPDR. This obvious lack of pericytes leads to the formation of micro aneurysms[63]. The presence of relative hypoxia in retinal vessels leads to the activation of growth factors like VEGF, PDGF and placental growth factor[61].

In nephropathy ,acute hypertrophy of the glomeruli occurs in the initial stages of diabetes, this along with the increase in mesangium leads to gross decrease in the filtration area. Kimmelstiel and Wilson[64] was the first to demonstrate the thickened GBM in diabetic kidneys. An elevated intra glomerular pressure and permeability leads to the excretion of proteins of 44 -150 kDa .the increased GFR and proteinuria in initial stages of DM can be reversed with intensive insulin therapy.[65]

Peripheral neuropathy is quite common in diabetics and is present in > 50 % of individuals. The abnormality can be accounted to changes in the neurons secondary to hyperglycemia or ischaemia produced by reduced neuronal blood flow[66-67]. Narrowing of capillary lumen and activation of protein kinase C has been demonstrated.

Changes in microvessels of the heart can be held responsible for the increased occurrence of chronic heart failure in diabetics. The reason behind this increased incidence is mostly diastolic dysfunction and reduced formation of collaterals following myocardial ischaemia(68) . The relative density of capillaries in myocardium of patients post infarction was reduced in diabetics than the normal control subjects. The cardiac neovascularisation and collateral formation is dependent on the homeostasis of multiple pro and anti angiogenic factors (VEGF, PDGF,FGF, angiopoeitins ,etc) . An imbalance in this milieu is evident in diabetes. Accumulation of extracellular matrix can lead to cardiac fibrosis thus resulting in diastolic dysfunction.

METABOLIC ALTERATIONS AND ITS INTRACELLULAR CONSEQUENCES

Hyperglcemia is considered to be the prime factor for the development of vascular complications ,however the evidence proves to be contradictory as there is no apparent reduction atherosclerotic complications in patients with lower blood glucose levels. The abnormalities in diabetes are attributed to the abnormally high uptake of glucose in the cells and elevated intracellular glucose concentrations. This is avoided in tissues exhibiting insulin resistance. Thus IR seems to be of beneficial effect by reducing intracellular concentrations of glucose. Thus the next step was that organs incapable of this mechanism of IR where prone to the complications of diabetes (111). This is seen in endothelial cells which express GLUT-1 receptors (they do not respond to insulin) and lack GLUT 2-5. In the setting of hyperglycemia the smooth muscle cells of the blood vessels are capable of down regulating GLUT-1 however the endothelial cells are incapable of doing so , thus making them more susceptible to complications.

INTRACELLULAR REDOX STATUS

The imbalance between the reactive oxygen species and antioxidant pathways result in oxidative stress .Abnormal metabolism of glucose or defect in the enzymes not associated to glucose metabolism is considered to produce this imbalance. Glutathione in its reduced form is found to be depleted in the

diabetic population (119).Organ systems developing microvascular complications are found to be affected by oxidative stress and antioxidant therapy helps in the prevention of organ damage. Cellular dysfunction is produced by means of production of advanced glycosylated end products, breakage of DNA, PARP activation and promoting other stress induced pathways ultimately leading to apoptosis.

ATHEROTHROMBOSIS – EVOLVING CONCEPT

Atherothrombosis, is defined as disruption of atherosclerotic plaque with super imposition of thrombosis. It's the leading cause of mortality. Atherosclerosis is a widespread systemic process and starts early in the first decade of life progressing asymptomatically into adult life. It clinically manifests as CAD (coronary disease), cerebrovascular disease,transient ischaemic attack (TIA),stroke, and peripheral arterial disease . It's often considered as a single pathological entity affecting various vascular territories. Atherothrombosis is a diffuse process affecting the arterial treeof systemic vasculature .The tunica intima of both medium and large sized arteries is involved (the tunica intima of the aorta, coronary vessels, carotid arteries and peripheral arteries as well). The plaques of atherosclerotic vessels are mainly

comprised of[68,69] : 1)extracellular rmatrix components (proteoglycans ,fibroelastic proteins, collagen) 2) phospholipids and esters of cholesterol ; 3) mononuclear inflammatory cells (T lymphocytes, macrophages, 4)pro thrombotic factors (platelets and deposits of fibrin).

Endothelial dysfunction , a widespread systemic pathological process is considered as the precursor to atherothrombosis. Two major hypotheses where proposed in the 19th century- von Rokitansky proposed the incrustation hypothesis and Virchow proposed the lipid hypothesis. They concentrated on lipid accumulation ,fibrin deposition and formation of extracellular matrix. It was Virchow who coined the term endarteritis deformans and linked inflammation to the process. The initial stages of the atherosclerotic process is characterised by chronic inflammation and retention of lipoprotein. The inflammatory process leads to the rupture of plaque and thrombosis [70]. All these hypotheses can be compiled together to form a single term – Atherothrombosis.

PHASES OF ATHEROTHROMBOSIS

According to the AHA classification the progression of the atherosclerotic plaque can be divided into 5 pathological phases with significant relevance clinically as well.

Phase 1 (early stage). These lesions most commonly seen in young adults are small and can be further divided into 3 types :

Type I : the lesions consist of foam cells formed from macrophages , containing droplets of lipids

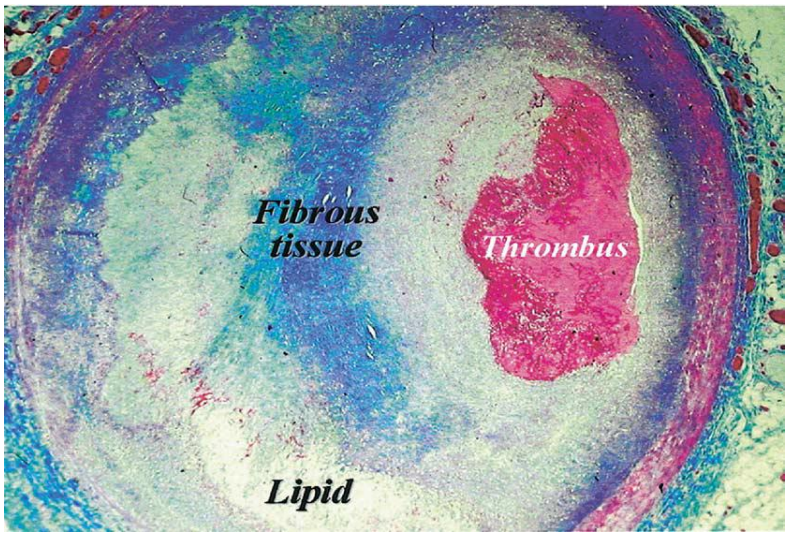
Type II : they are comprised of vascular smooth muscle cells, mononuclear cells (macrophages) and extracellular deposits of lipids.

Type III : they are made of connective tissue surrounding smooth muscle cells and deposits of lipid .

Phase 2 (advanced stage). In this stage though not essentially stenotic , are prone to rupture due to increased content of lipid , presence of inflammatory process and a relatively thin cap of fibrous tissue. They are further classified morphologically into two forms:

type IV: they are charecterised by confluence of cells which is rich in lipid derived from the extracellular compartment and normaly appearing intima forming the outer cap

type Va: lesions appear as an acquired fibrous cap covering extracellular lipid core.These lesions progress into the acute stages of phase 3 and 4



This is a cross section of coronary artey with an occlusive thrombus overlying an atheromatous plaque.

Phase 3. They are the complicated forms of type 4 lesions. They evolve from ruptured or eroded lesions, and form non-obstructive, mural thrombi. This process is usually clinicaly silent but on rare occasions present as angina[70].

Phase 4. they are also a complicated version of type 4 lesions, presenting as a thrombus which is fixed or that which causes reccurent occlusions. This stage of atherosclerosis has clinical manifestations and presents as an acute coronary syndrome, certain lesions however remain silent [71]. About 2/3 rd of acute coronary syndromes are produced when occlusion in produced by thrombus in a plaque that is not stenotic

However in the remaining one third of conditions , are produced by thrombus occluding a plaque that is stenotic .[70] In the third and fourth phases

of plaque formation the connective causes organisation of the lesion leading to a thrombus that is stenotic or produces occlusion or complete fibrosis.

Phase 5. They are divided into two types Vb which is calcific

And Vc which is fibrotic. Both these lesions may produce angina chestpain .However,if there is occlusion and an episode of myocardial ischaemia the tissue is then protected by the formation of collateral vessels.in such a case the lesion is found to be clinicaly silent.

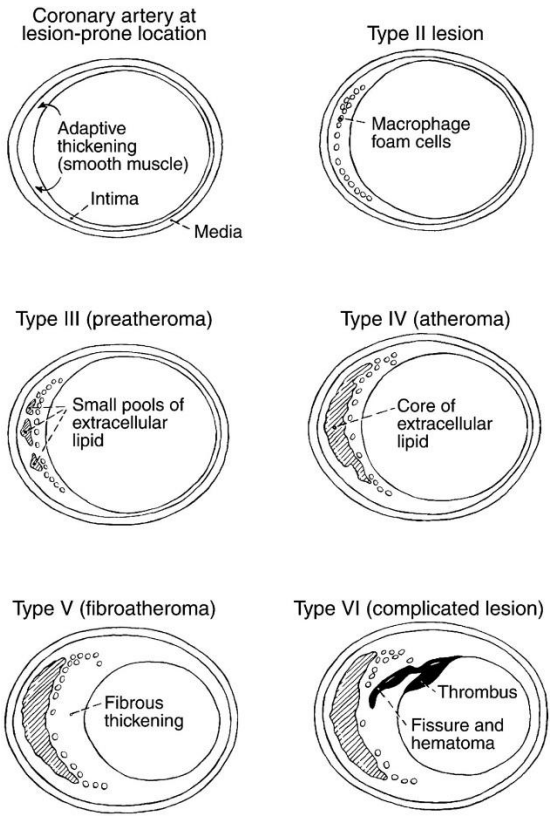


Figure 9 atherosclerosis stages

EARLY ATHEROTHROMBOSIS

ENDOTHELIAL DYSFUNCTION

The endothelium is considere as a dynamic structure, having autocrine and paracrine functions. It regulates anti inflammatory ,mitogenic, and contractile processes of the vasculature, also produces regulation of the hemostatic activities happening inside the vascular lumen . The single most vital molecule responsible for the entire process is nitric oxide (NO). Dysfunction of the endothelium leads to reduction in synthesis of NO, which paves way for the lipoproteins in circulation to enter and undergo oxidation. Lack of NO also favours the diapedesis and internalization of macrophages, favours inflammatory processes, proliferation of smooth muscle cells and deposition of ECM, also favouring vasoconstriction all favouring the formation of intraluminal prothrombotic state[(72)].

Dysfunction of the endothelium is universally considered as the earliest step atherothrombosis. An alteration in the blood flow pattern either a reversal of flow or occurrence of shear stress of oscillating type (mainly at the areas of vascular bends or bifurcations) result in this dysfunction of endothelium. Apart from the shear stress, the presence of biohumoral risk factors like dyslipedemia , advanced glycation end products ,the irritants found in tobacco smoke , immune mediated complexes and vasoactive amines in

circulation contribute to endothelial dysfunction. The endothelium responds to the shearing stress by way regulating the suppression or activation of various genes .thus it leads the increased formation and release of cellular adhesion molecules (CAMs) from the family of selectins (E selectin and P-selectins). They in turn accelerate the monocyte margination and its adhesion to the surface of the activated endothelium. These proteins of cell adhesion in the vascular compartment favour the internalization of mononuclear cells adhered to the vessel wall into it thus paving the way to atherogenesis. Clinical studies have demonstrated an association between the elevated cellular adhesive molecules and an increased risk for cardiovascular derangements.

PROTEOGLYCANS AND LIPOPROTEIN TRANSPORT

The entry of Low-density lipoproteins into the tunica intima of the vessel wall is facilitate by the interaction between the proteins of extracellular matrix (proteoglycans, collagen, fibronectin)and apo B lipoprotein fraction (49) . Proteoglycans are biochemically composed of a core component formed by protein and a glycosaminoglycan chain (a long chain carbohydrate). These proteoglycans and the proteins of ECM are present between the internal elastic lamina and the endothelial cell's basement membrane . The critical step in early atherosclerosis is the interaction between oxidized LDL and proteoglycans .the other lipoprotein playing an important role in atherosclerosis

is HDL. Its primarily called as the anti atherogenic lipoprotein, HDL brings about reverse cholesterol transport ,i.e transport of cholesterol from the arterial wall, especially from lipid-laden macrophages inspite of its protective effects, individuals with high HDL levels can still present with acute coronary syndromes, this can be attributed to the elevated levels of HDL3 rather than HDL2.

Innate and adaptive immune response to auto-antigens

Inflammation is found to play an important role in atherothrombosis and the focus has now shifted on the immune system. innate and adaptive immune responses greatly influence the development of atherosclerosis [(73)]. The first step in the immunological response to organisms is by innate immunity .The toll-like receptors(TLR) and scavenger receptors form the critical receptors of innate immunity in atherosclerosis [(74)].

In the first step of this immune process there occurs the uptake of LDL in its oxidized form by SR-A and CD-36 (the scavenger receptors) and then the production of foam cell from a macrophage.[(74)]It results in the activation of NF κ-B a nuclear transcriptional factor, which in turn initiates a strong chemo attractant cycle leading to migration of monocyte and macrophage /foam cell formation .These activated macrophage/foam cells produce cytokines

which activates the neighbouring smooth muscle cells, leading to formation of extracellular matrix and fibrosis .

The innate immunological response in its second step, the Toll like receptors play a significant role. Stimulation of TLR4 results in activation of NF-kappa-B and elevated synthesis mRNAs of numerous cytokines [(75)]. Activation of TLR4 in adventitia augments the formation of neo intima. TLR4 is involved both in the initiation, progression and expansive remodelling of atheromatous plaque.

The adaptive immunity has a more organised response , however it takes several weeks to initiate the response is characterised by generation of immunoglobulins, T and B cell receptors capable of recognising foreign antigens.

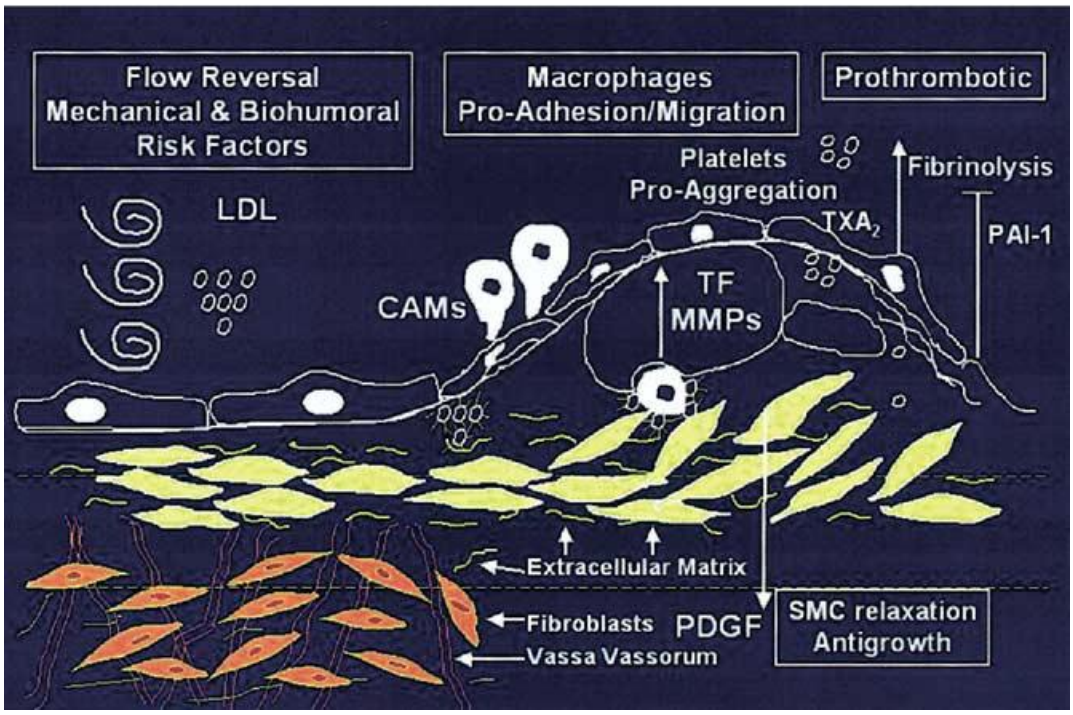


Figure 10 endothelial dysfunction

MECHANISM OF CALCIFICATION

Calcification in atherosclerosis is due to deposition of both hydroxyapatite and organic matrix .This organic matrix is comprised of type I collagen and other non-collagenous bone-associated proteins (NCPs) [(76)]. Collagen produces crystal accumulation ,this potentiates mineralization occurring inside the vesicles of extracellular matrix. Thus dystrophic calcification is considered now as an active, organised entity rather than a mere passive calcium accumulation .

The important non collagenous proteins, associated with calcification of vasculature include osteopontin (OPN), osteonectin,

osteoprotegerin, and matrix Gla protein. The most extensively studied among these is osteopontin (OPN). OPN was found to be expressed in atheromatous macrophages , adventitial cells and the smooth muscle cells of the aortic wall.

ADVANCED ATHEROTHROMBOSIS

The prolonged exposure to a pro-atherogenic milieu increases the chemotaxis of mononuclear cells , lipid accumulation, formation of necrotic core and fibrous cap – thus progressing to advanced atherosclerosis. Expression of metalloproteinase and inflammation results in plaque rupture occurring at the shoulder of large plaques with high lipid content .Newer structural and functional alterations have been identified, eccentric growth of the plaque with an enlargement of the vessel wall which is compensatory. This process is known as vascular remodelling, its associated with neovascularization vasa vasorum resulting in an expansion of the lipid core and occurrence of haemorrhage within the plaque.

ECCENTRIC VASCULAR REMODELLING

The growth of an atheroma eccentrically within the inner constituents of the vessel wall prior to formation of luminal obstruction is also considered as vascular remodelling. The study by Glagov et al [(78)], has found

remodelling as a consistent factor in atherosclerotic lesions of the acute coronary syndromes.

The remodelled atherosclerotic plaque is found to consist of a core rich in lipids, minimal smooth muscle cells with numerous macrophages infiltrating the plaque .Rather than the concentrical growth within the lumen as expected the eccentrical growth triggers crucial alterations in the tunica media and adventitia of the vessels. The hyperactivity of metalloproteinases destroys the Internal elastic lamina, forming the crucial step of remodelling. Recent studies have demonstrated the break in the IEL as an independent

predictor of rupture of plaque . Furthermore tunica media showed increased levels of inflammation, fibrosis, and atrophy. The non-ruptured plaques had less of inflammation of the adventitia than that of the plaques undergoing rupture [77].

VASO VASORUM NEOVASCULARISATION

The adventitial vasa vasorum is responsible for nourishment of blood vessels by way of diffusion of oxygen from the vascular lumen . When the thickness of the vessel wall is greater than the appropriate distance required for effective oxygen diffusion then there is a proliferation of vasa vasorum into the internal layers of the vasculature (where it is usually absent). This

proliferation of vaso vasorum is driven by cytokines produced by the Macrophages which are in turn attracted by oxidized LDL.

There are 2 anatomical forms of vasa vasorum; the first-order which proceed in a longitudinal fashion into the vessel lumen and the vasa vasorum of second-order are circumferentially arranged around the wall of the vessels.

However, in the setting of atherosclerosis the vasa vasorum proliferates, causing a significant neovascularization of the tunica media and they are seen proliferating in the direction of the atheromatous lesion rich in lipids. The newly formed microvascular content was higher in the plaques of the human aorta undergoing rupture than those of the unruptured ones.similarly recent studies point to an increased content of new vessel formation in the atheromatous vessels of the diabetic population. [78] the plaques with a reduced lipid content and more of fibrocalcific lesion are called as regressive lesions. These regressive lesions had a relatively low neovascular content than the lipid rich ones . Thus the vasa vasorum are considered to play a prominent role in the reverse lipid transport. During reverse lipid transport the microvessels and the outer layers of the vascular wall undergo the aforesaid process of regression. This has been proven in animal studies with plaque regression. .

PLAQUE RUPTURE

Two mechanisms are attributable to plaque rupture they may act independently or in conjugation to produce rupture. Firstly, it is attributed to the to physical forces and occurs most commonly in the thinnest areas of the the fibrous cap ,the site which is heavily infiltrated by foam cells, and thus the most weakest .For eccentric plaques, this weakest spot occurs at the shoulder or at a point between the vessel wall and the plaque[79]. Vulnerability of the plaque to rupture is said to depend on multiple components. these include the stress on the vessel wall exerted circumferentialy and cap fatigue; the consistency ,size and location of the core of atheromatous lesion; and finally the characteristics of blood flow ,most importantly the flow impact on

the proximal part of the plaque (i.e. the plaque angulation).

The second of the mechanisms involves a process which occurs inside the plaque leading to its rupture in the macrophage and mast cell rich

. These macrophages and mast cells within the plaque initiate proteolysis and phagocytosis resulting in the degradation of ECM. According to a human study the quantity of few matrix metaloproteinases and their inhibitors correlate with the extent of atherosclerotic process . The macrophages in order to shield the blood vessel wall from lipoprotein accumulation undergo apoptosis. [80] The apoptosis produces membrane microparticle shedding and the consequent exposure of cell surface phosphatidylserine(the prime contributor for

formation of thrombosis after rupture of plaque. [80] It is now clear that apoptosis is the common link between thrombosis and inflammation.

PLAQUE DEPENDENT THROMBOGENIC SUBSTRATE

The arterial site thrombogenicity is determined to the exposure of a thrombogenic substrate. Lipid-rich plaques are found to be the most thrombogenic among all others. Tissue factor, is the glycoprotein that initiates the extrinsic clotting cascade and plays a key role in regulation of hemostasis,coagulation, and thrombosis . Tissue factor forms a complex with factors VII/VIIa with immense affinity; these Tissue Factor/VIIa complex in turn activate

factors IX and X, which ultimately results in generation of thrombin .The presence of TF in apoptotic macrophages, reiterates its role in ACS .In addition, by inhibiting the TF using r-tissue factor pathway inhibitor resulted in decreased formation of acute thrombus among the plaques rich in lipids . This further substantiates the vital part played by Tissue Factor in thrombosis formation in coronary arteries.

RHEOLOGY AND THROMBOSIS

The other important factors determining the thrombogenic potential at the site of arterial disruption is the extent of of stenosis produced by the plaque rupture and the thrombus overlying it. Thus, degree of narrowing modulates the platelet deposition after plaque rupture . The geometrical changes increases deposition of platelet , however a sudden increase in thrombus size may accentuate the stenosis and lead to thrombotic occlusion.

SYSTEMIC PROCOAGULANT ACTIVITY

The two major pathways accountable for pro-coagulant activity are factors affecting the coronary arteries and the tissue factor in circulation. Atherogenic dyslipedemia smoking, hemostasis, hyperglycemia and various other factors are linked to the elevated blood thrombogenicity As seen earlier levels of increased LDL cholesterol are found to increase the thrombogenic potential of blood and favours the proliferation of thrombus under specific rheological conditions . Smoking increases the release of catecholamine, potentiates activation of platelets and elevates the fibrinogen levels[81]. The increased incidence of sudden cardiac deaths can be attributed to catecholamine -dependent effects .The platelets of the diabetic people are hyper-reactive and have increased aggregation potential and also display an array of activation-dependent adhesion proteins .The activation of interactions between leukocyte and platelet results in the secretion of tissue factor and activation of thrombin.

The levels of Tissue Factor in circulation is found to correlate with increased thrombotic potential in people with Acute coronary syndrome and chronic CAD.

There has been a demonstrated increase in microparticles with increased TF activity originating from monocytes, this indicates the existence of a causal relationship between pro coagulant activity of the plaque and the microparticles shed from the membrane surface. Elevated levels of CRP has also been observed, whether the higher levels of CRP are due to the inflammatory mediators of the blood or that released from the plaque is still unclear. Whether it is a biologically active element in the process of plaque development or thrombus formation is also questionable. Recent experiments indicate to CRP being the cause for activation of monocyte and endothelial cells in vessel.

DETECTION OF ATHEROSCLEROSIS

Imaging techniques help in detecting subclinical pathologies and may also serve as a surrogate technique thereby is in the improvement or in complementing the assessment of cardiac risk. Ultrasound examination is used to measure the of the thickness of aorta and carotid arteries , as well as qualitative and quantitative examination of the atherothrombotic plaques. A heterogenous plaque which is hypoechoic on ultrasound is indicative of lipids

or haemorrhage within the plaque. Likewise a plaque that is homogenous and hyperechoic indicates fibrosis..The intima-media thickness of large- and medium-size arteries is measured by Real-time B-mode ultrasound, especially the carotid, femoral, or radial. Presence of carotid and aortic atherosclerosis indicates the concomitant presence of coronary artery involvement . A strong correlation was noted between AHA classification and the MRI images. The variation in the maximum thickness of coronary vessel wall of normal as well atherosclerotic arteries was found to be significant statistically,This was done by performing high resolution black-blood MRI

The blood pressure is measured in both the brachial arteries and the two posterior tibial arteries as well as the dorsalis pedis, for calculation of ankle-brachial index. Ankle-brachial index values when low (<0:90) are suggestive of a peripheral arterial disease. Furthermore,progressively decreasing trend of ABI values points to a critical obstruction. The presence of a generalised systemic atherosclerosis is indicated by the lower levels of ankle brachial index.

BIOMARKERS FOR ATHEROSCLEROSIS

A selected group of biochemical parameters are said to indicate the presence of atherosclerosis, however their specificity is questionable.there is constantly increasing evidence that minimalistic elevation of CRP is predictive of adverse vascular processes in asymptomatic , healthy adults.Biomarkers in atherothrombosis can be grouped as Inflammation markers – comprising of interleukins , CRP, amyloid A, CD40 ligand, cellular and vascular adhesive molecules and the second set as markers of thrombosis – v WF ,fibrinogen, PAI- 1and prothrombin fragment 1 and 2.[82]

Atherothrombosis is a widespread ,systemic disease where the main players are deposition of cholesterol,inflammatory process and formation of thrombus. This process is clinicaly not evident in the early stages and subjects are asymptomatic and presents dramatically with the formation of thrombosis and its complications.the five main stages of the disease process is dealt in detail in subsequent pages.advanced stages and coplex lesions are characterised by the presence of formation of new micro vessels and hemorrhages within the plaque. The circulating Tissue factor, considered the strongest trigger of the coagulation cascade, plays a vital role in determining the thrombogenic potential of the plaque.The microparticles of tissue factor in circulation are found to be in association with the monocytes thus completing the link between inflammation,rupture of plaque and thrombogenicity.

PLATELETS

Platelets are discoid, fragments of cell lacking a nucleus that are formed by cleaving from the bone marrow megakaryocytes .Maintaining the hemostasis , formation of the primary hemostatic plug (which is the first step following endothelial damage) and providing an active surface for the recruitment and concenteration of coagulation factors are the main functions of the platelets. A brief overview of the hemostatic processes is required for better understanding of the pathological basis of pro thrombotic state in diabetes.

Following a vascular insult the platelets come in contact with the extra cellular matrix components and its proteins , they then undergo change in shape, adhesion to the endothelium, secretion and the release of granular content and finally aggregation .

- The adhesion of platelets to the extracellular matrix is mainly regulated by the von Willibrand factor , which bridges the platelet surface proteins mainly the Gp-Ib to the collagen. Though the platelets have the capacity to adhere to various other molecules in the ECM their interaction with von

Willibrand factor is essential to counteract the high shearing forces of the blood column.

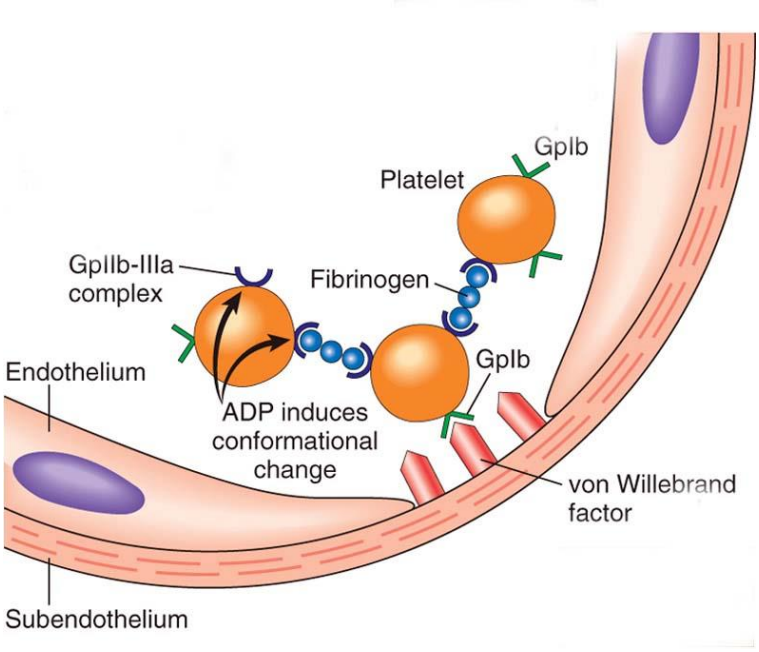


Figure 11 adhesion and aggregation of platelets

Adhesion is immediately followed by the secretion or release of both the type of granules in the platelets.The platelet cell surface receptors undergo binding with various agonists leading to phosphorylation of the intracellular proteins and produces the degranulation of platelet contents. The components of the dense granules of pletelets namely calcium and ADP (produces a strong activation of aggregation) play a vital role in the coagulation cascade and their release

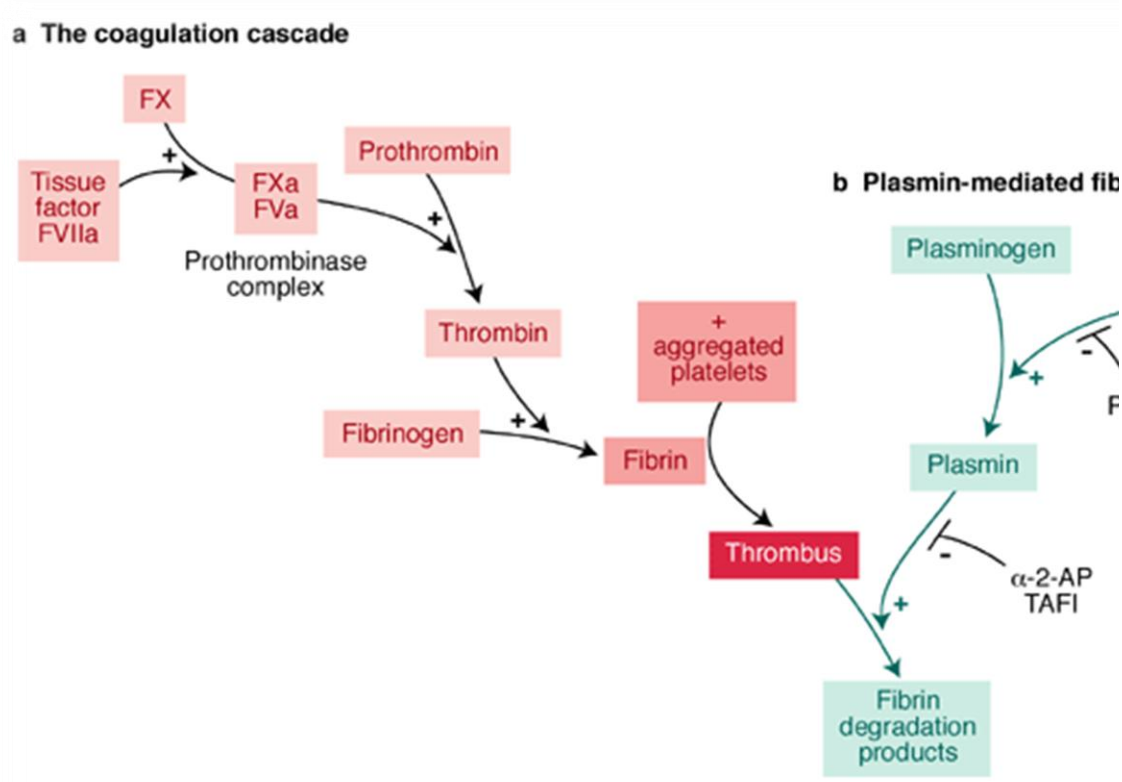
therefore forms a critical step. The platelets after activation express negatively charged phospholipids (especially phosphatidylserine) on their surfaces. They inturn bind to calcium and act as important sites of nucleation required for the organisation of coagulation factors and its complexes.

Aggregation :

Adhesion and release of granules is followed by aggregation of platelets. Apart from ADP thromboxane A_2 a potent vasoconstrictor is a critical stimulus that is required for the amplification of platelet aggregation, all of these mechanisms result in the formation of the primary hemostatic plug. These initial processes are reversible , but the simultaneous activation of the coagulation cascade produces thrombin, which results in stabilisation of the primary hemostatic plug through 2 mechanisms. In the first process thrombin binds to a pecific activated receptor on the platelet surface , this is compounded by the effects of thromboxane A2 and ADP propagating firther aggregation.the next step is the contraction of platelets resulting in a stonger and tight bond forming the secondary hemostatic plug.later fibrin is formed from fibrinogen leading to the completion of process.

Fibrinolysis

Alongside the coagulation cascade a fibrinolytic process is also initiated that regulates the clot size. Plasmin is the main moderator of this fibrinolytic process , wherein it is responsible for breaking down of fibrin from its complex polymerised form. The end products of this fibrin breakdown have a weak anticoagulation action. The enzymatic breakdown of the precursor plasminogen results in formation of fibrin, this can be either due to factor XII mediated pathway or through the plasminogen activators . the key molecule among the plasminogen activators is the tissue plasminogen activator or tPA. Its released by the endothelium and is highly efficient when bound to fibrin.



Summary of the coagulation and fibrinolysis cascades

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The activation of the anti fibrinolytic pathway by the endothelium results in finetuning this balance. This is achieved through the action of plasminogen activator inhibitor (PAI); which causes the inhibition of tPA and thus produces a pro coagulant effect. Thrombin and few other cytokines facilitate PAI production, thus explaining the increased thombotic potential in inflammatory states.

THE EFFECTS OF DIABETES ON PLATELETS

Insulin resistance acts as a common denominator in all individuals with type 2 diabetes. This insulin resistance coupled with defects in insulin action creates a milieu of platelet dysfunction conducive to the complications of diabetes, both micro and macrovascular. The resistance to insulin stimulated uptake of glucose which forms the common precursor to diabetes and its vascular complications is considered as a multisystem disorder affecting various metabolic and cellular pathways. Genetics, advancing age , physical inactivity and obesity are the contributory factors to insulin resistance.

Defining insulin resistance merely as a defective regulation of glucose utilization grossly undermines the numerous other myriad effects produced by defective insulin action .The more appropriate definition would be "the metabolic state in which the measured tissue response to insulin is less than that expected for the apparently available insulin." This response is applicable to metabolic fuels ,the effects of insulin on smooth muscle, endothelium, platelet function, erythrocyte function and neuropeptide secretion .prothrombotic state is a recent addition to the insulin resistance syndrome .the pattern of coagulation dysfunction in IR is characterised by increased propensity for thrombosis and retardation of thrombolysis. Elevated levels of Plasminogen

Activator Inhibitor 1 (PAI -1), fibrinogen and intrinsic platelet abnormalities are seen in Insulin Resistance.

INSULIN RESISTANCE AND PROCOAGULANT STATE

The coagulation cascade in IR is associated with abnormalities of platelet aggregation , adhesion , abnormal levels of vWF , factor VIII, thromboxane ,tissue plasminogen activator, fibrinogen and PAI-1. The European Concerted Action on Thrombosis Study identified the association between the components of IR and hemostatic factors. Higher levels of fibrinogen,PAI-1,vWF,tPA and prolonged clot lysis time was found to correlate with elevated levels of circulating insulin.

There was a strong and consistent correlation between high PAI-1 and increased insulin levels and decreased insulin sensitivity.[83]Insulin ,pro-insulin and split products of insulin also correlated with high PAI-1 levels [83]. Likewise pro-insulin was associated with elevated fibrinogen levels in subjects with IR. Thus the two prime determinants of plaque formation (fibrinogen, PAI-1) are related to high circulating levels of insulin and its precursors.

DIABETES, PLATELET, ATHEROSCLEROSIS

An increased intravascular thrombus formation and depressed fibrinolytic activity contribute to atherosclerotic plaque production in diabetes mellitus. One of the metabolic derangements of insulin resistance syndrome is atherogenic dyslipedemia- increased VLDL and small LDL particles with decreased HDL .This atherogenic phenotype of IR is found to precede the occurrence overt diabetes by many years. The higher levels of PAI-1 is found to have a positive correlation with BMI and fasting plasma insulin in type 2 diabetic individuals .

Similarly elevated triglyceride levels had a positive correlation in non-diabetic obese subjects. Physical activity and weight reduction brings down hyperinsulinemia and IR which in turn reduce PAI-1 levels thereby increasing fibrinolytic activity. This explains why vascular complications are common in obese than non-obese diabetics[(84)]. Thus the reason for reduced fibrinolytic activity in diabetic id due to elevated PAI-1[(88)] which inhibits the breakdown of plasminogen to plasmin.

PLATELET AND EDOTHELIAL CELL INTERACTION

Vascular damage endothelial injury, plaque rupture leading to aggregation and adhesion of platelets at the site , form the critical step in macrovascular complication of atherogenesis.The endothelium of healthy

individuals constantly release prostacyclin (PGI 2) and NO which prevents platelet aggregation and adhesion. At the site of endothelial injury in response to plasma thrombin , serotonin released from platelets, bradykinin, PDGF, IL2 and ADP there occurs an increased production of PGI 2 and NO. This negative feedback loop limits the formation of platelet plug and de-endothelization.

Normally prostacyclin binds to G protein receptor on the platelet surface leading to stimulation of adenylate cyclase enzyme. G protein inhibitory (G1) for adenylate cyclase is linked to α2 adrenergic receptor, which is capable of binding to epinephrine. NO directly diffuse across the plasma membrane and activates guanylate cyclase enzyme. Thus both the inhibitory pathways producephosphorylation of c-AMP and c-GMP dependent protein kinases resulting in inactivation of platelets and thus prevents their aggregation.

Studies have demonstrated a reduced synthesis of vascular PGI2 and reduced release of NO in diabetic models thereby contributing to increased platelet activity. Apart from the reduction in synthesis ,a reduction in sensitivity of platelets to anti-aggregatory molecules have also been demonstrated in diabetics. Platelets respond to a lesser degree to Prostacyclin and Nitric oxide in diabetic individuals.[84]

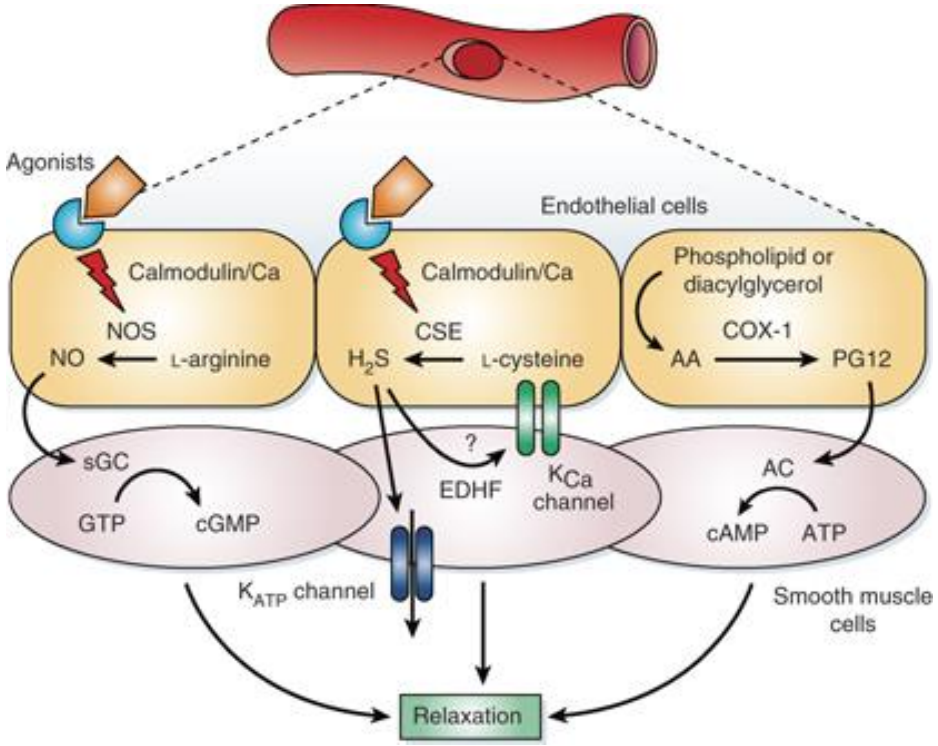


Figure 12 endothelial mediators ,NO,PGI2

Various mechanisms have been proposed for this reduced sensitivity of platelets, the concepts are discussed below.

Impaired PGI 2 receptor activity

- Modesti et al [(85)] showed that platelet in diabetics had normal levels of PGI2 receptors. Thus the problem was considered to be further downstream
- Livingstone et al[(86)] found a decrease in the levels of G1 (the glycoprotein for inhibitory pathway) in platelet membrane of diabetics. Thus it resulted in reduced stimulation of adenylate cyclase in response to

stimulation by PGI2 .

❖ Bon et al [(84)] demonstrated a decreased c-AMP response in obese insulin resistant individuals tha non-obese subjects. The exact underlying mechanism is still unclear.

❖ Another study demonstrated a decrease in level of G protein stimulated platelet phospholipase C activityresulting in enhanced aggregation in response to thrombin.

❖ In type 2 diabetics ,genetic anomalies lead to inactivity of G proteins , produced by alteration in level of expression or sequence of genes.

This could be explained by two mechanisms:

- Mutation of G protein genes
- Dysregulation in the process of posttranslational modification of G protein

Diabetes mellitus being a disease with genetic predisposition ,there is a highincidence of post translational modification of protein leading to abnormal functioning or inappropriate localization of protein.[(86)]

❖ von Willibrand factor a glycoprotein constituent of the factor eight complex produces platelet activation. This vWF binds to platelet GpIb and IIb-IIIa receptor on the platelet membrane and promote clumping. Increase levels of vWF and increased activity has been documented in diabetics experimental studies.[(86)] thus indicating the presence of endothelial damage. Similarly

insulin has shown to deplete the levels of von Willibrand factor in experimental diabetic models.this mechanism also contributes the procoagulant state in diabetes mellitus.

INTRINSIC PLATELET ABNORMALITY

There are no clear studies which indicate whether the platelet dysfunction in diabetes is due to intrinsic platelet defect or as an impact of circulating factors on platelet function. [85].

The platelet sensitivity to ADP, epineohrine and thrombin is enhanced in human diabetics- which is independent of arachidonic acid and ADP pathways.This hyperaggregability was not reversible with insulin.

In the initial studies: increased thromboxane A2 (TXA2) and prostaglandin E2 caused platelet hyperaggregability which was reduced by inhibition of cyclooxygenase enzyme.it was later found that platelet aggregation did not correlate with thromboxane A2 synthesis , indicating it's a multifactorial process.

- Thus events occurring prior to eicosanoid production like IONOSITOL PHOSPHOLIPID turnover and CALCIUM release was focussed as a possible mechanism for platelet sensitivity. Elevated levels of calcium and suppressed intracellular magnesium was seen associated with platelet aggregation in

diabetic.[86]The increase sensitivity of platelets to primary agonists like thrombin ,collagen ,ADP is accompanied by increased phosphoinositide turnover. [87]

- Similarly the basal and agonist(collagen) stimulated calcium levels where found to increase in platelets of diabetics than control subjects.

ADVANCED GLYCOSYLATION ENDPRODUCTS

Advanced glycosylation end products – produced by non - enzymatic reaction between amino group of proteins and genes are found to be accumulated in tissues of diabetics.

The glycosylation of membrane proteins in platelets in turn reduce the membrane fluidity in diabetic subjects..This effect on membrane fluidity results in platelet hyperfunction. Likewise the glycosylation of subendothelial proteins reduce the nitric oxide levels and in turn lead to prothrombotic state.

The atherogenic dyslipedemia triad of DM also affects the membrane fluidity. The LDL of diabetic persons had greater sensitivity and resulted in increased aggregation compared to control subjects and this significantly correlated to levels of glycosylation.[88]

The excess of free radicals production as a result of oxidative stress and

lipid peroxidation in diabetics result in decrease in prostacyclin and nitric oxide, thus amounting to platelet reactivity.

ELEVATED GLYCOPROTEIN EXPRESSION

Evidence points towars increased expression of glycoprotein Ib, IIb-IIIa on platelets of diabetics. Thusthese glycoprotein acts on the receptor for vWF , fibrinogen and other adhesion proteins involved in platelet aggregation-thus platelet hyperreactivity.

DIRECT EFFECTS OF INSULIN

Platelet contains a functional insulin receptor which is capable of binding insulin and autophosphorylation. Insulin reduces the platelet response to the primary agonists , by downregulation of α2 receptors.Insulin also maintains the sensitivity of platelets to PGI2.

Ud vardy et el[88] demonstrated reduced number of insulin receptors and reduced affinity in diabetics.

Diabetes is a state associated with elevated platelet reactivity. Factors that contribute directly to greater platelet reactivity include metabolic abnormalities such as hyperglycemia andatherogenic hyperlipedemia, both insulin resistance (relative insulin deficiency) and absolute insulin

deficiencyalong with associated conditions such as oxidative stress, inflammation, and endothelial dysfunction. The increased platelet aggregability and adhesiveness in diabetes are due to the following:

Reduced membrane fluidity

Altered Ca+2 and Mg+2 homeostasis

Increased arachiodonic acid metabolism

Increased TXA2 synthesis

Decreased prostacyclin and NO production

Decreased antioxidant levels

Increased expression of activation dependentadhesion molecules

(e.g. GPIIb/IIIa, p-selectin)

MEAN PLATELET VOLUME

MPV is an indicator of the average size and activity of platelets. Platelet size and volume depends on the circumstances of their production in the marrow. MPV is not related to aging of platelets in the circulation. Platelet parameters are very stable in most patients.

Normal range of mean platelet volume in healthy individuals is 8.9±1.2 fl. MPV is higher in type 2 diabetic patients than in non-diabetic

patients in the range of 10.1±1.2 fl.

Platelets when larger are younger, increasingly aggregable and more reactive. The larger platelets contain denser granules, secrete more β-thromboglobulin, serotonin, and thromboxane A2 than their smaller counterparts. these changes produce a pro-coagulant state and cause thrombotic complications. This conceptualises a relationship between the platelet function mainly Mean platelet volume and vascular complications in DM .It implies that the changes in MPV reflect the state of thrombogenesis . Elevated MPV is an emerging marker for atherothrombosis which plays a pivotal role in the vascular complications of DM .

MATERIALS AND METHODS

This study was performed at Government Royapettah Hospital from april 2014 to to September 2014.

STUDY DESIGN AND PATIENT SELECTION

This is a cross sectional , comparative study performed by selecting 50 patients attending the diabetology OPD in Government Royapettah Hospital .Patients with type 2 diabetes (confirmed by the ADA criteria) for a more than a year and with good compliance to treatment for a minimum duration of 6 months were selected by simple random sampling technique . The study protocol was approved by the Ethical Committee for research in Government Kilpauk Medical College .

INCLUSION CRITERIA:

- Confirmed cases of type 2 diabetes on insulin therapy for minimum of 6 months
- Confirmed cases of type 2 diabetes on treatment with oral hypoglycaemic agents for a minimum of 6 months

EXCLUSION CRITERIA

- Patients with type 1 diabetes
- Patients with abnormal platelet counts
- Patients taking anti-platelet medications
- Patients with chronic kidney disease

After obtaining informed consent from the study subjects, a detailed history was elicited including history about the duration of diabetes and affected family members, treatment history .All the members were then subjected to a thorough clinical examination by obtaining , height, weight, systemic examination along with the examination of fundus. The following tests were performed on the study subjects .

Fasting blood glucose

Postprandial blood glucose

HbA1C

Blood urea

Serum creatinine

Urine for albumin and sugar

Complete blood count (including platelet count)

Mean platelet volume

The fasting and postprandial blood glucose measurements were made following a standardized test meal.

STANDARDIZED MEAL TEST

A standardized mixed meal was given to all the patients after an overnight fast. The meal consisted of three idlis and a standard serving of sambar and a standard serving of coconut chutney. The total energy content of the standard meal was 9 Kcal/kg, with 60% of the total energy from carbohydrates, 20% of the energy from fat and 20 % of the energy from proteins. Blood glucose levels were then measured.

Complete hemogram and mean platelet volume was performed by cellenium 19 cell counter method.

Data analysis was performed by SPSS using Student's t test and Pearson Correlation

OBSERVATION AND RESULTS

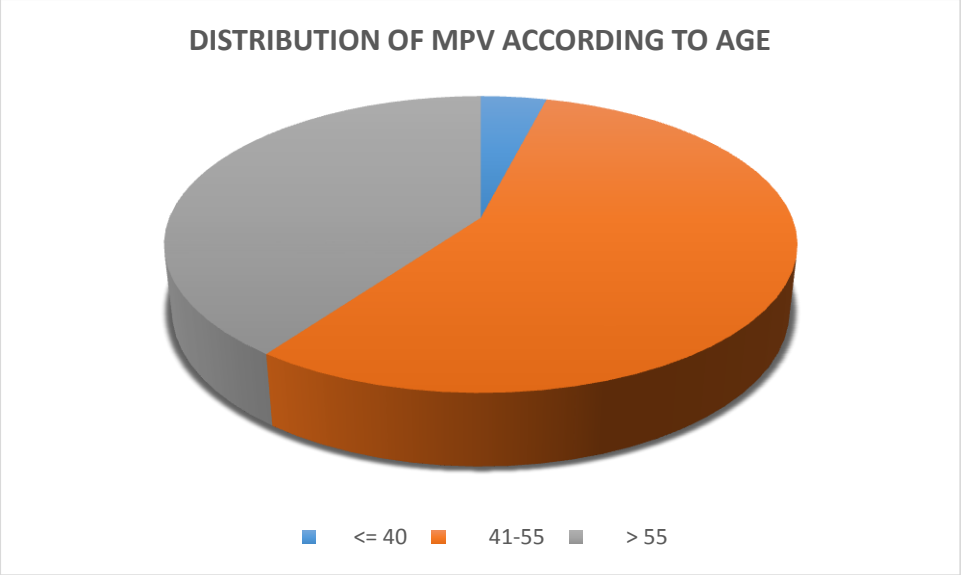
The study population of 50 analysed and the results were tabulated as follows.

THE DISTRIBUTION OF STUDY POPULATION ACCORDING TO AGE AND CORRELATION WITH MPV

Age (yrs)	N	Mean	Std. Deviation	P value
<= 40	2	10.800	.0000	.715
41-55	28	10.886	.5038	
> 55	20	10.770	.4692	

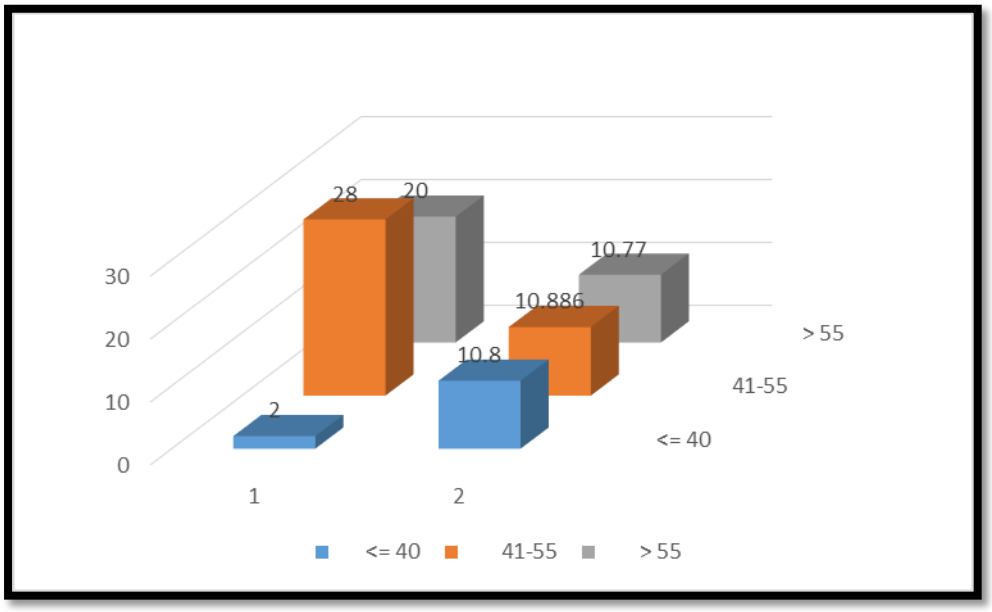
In the study population of 50 the mean age was 52.56 years.

- 20 subjects where above 55 yrs of age and had a mean MPV of 10.770 fl
- 28 of them where in the age group of 41- 55 years and the mean MPV of this age group was 10.886 fl and standard deviation of ± 0.5038.
- And 2 subjects where below 40 years of age and the mean MPV in this age group was 10.80 fl.



There was no statistically significant association between age and mean platelet volume in the study. (p value = 0.715)

Table 1 MPV with age wise distribution of study population



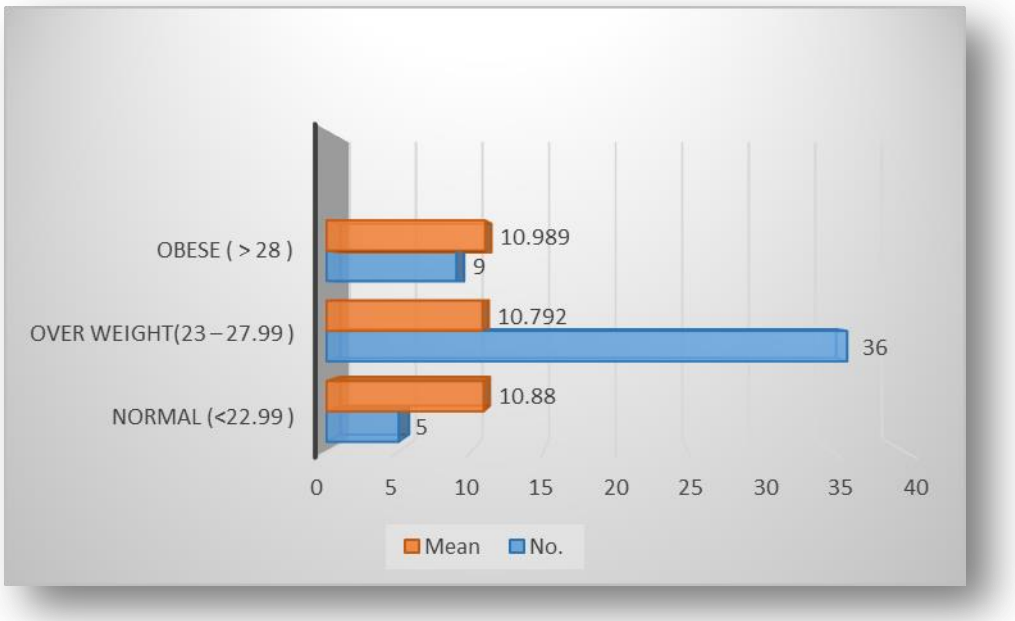
THE DISRTIBUTION OF STUDY POPULATION ACCORDING TO BMI AND ITS CORRELATION WITH MPV

BMI (Asian population)	N	Mean	Std. Deviation	P value
Normal (<22.99)	5	10.880	.3701	.539
Over weight(23 – 27.99)	36	10.792	.4795	
Obese (> 28)	9	10.989	.5372	

- In the study population 5 were classified as having normal BMI (for the Asian population) and the mean MPV of this group was 10.880 ±0.37 fl.
- 36 people in the study population were found to be overweight . the mean MPVvalue of this group was 10.792 ±0.4795 fl.
- 9 of the study subjects were classified under the obese group and their mean MPV was 10.989 ±0.5372 fl.

There was no significant statistical correlation between the mean platelet volume values and BMI.(p value = 0.539).

Table 2 MPV and distribution of study population according to BMI



DISTRIBUTION OF THE STUDY POPULATION ACCORDING TO DURATION OF DIABETES AND ITS CORRELATION WITH MPV

Duration of DM(yrs)	Number	Mean MPV	Std. Deviation	P value
< 3	20	10.675	.4655	.180
3-5	11	10.845	.3857	

5-10 > 10	13 6	11.046 10.900	.5238 .4858	

- In the study population 20 people are found to have DM for less than 3 years and the mean MPV of this group is 10.675 ±0.4655 fl.

- And 11 persons come under the 3-5 year group and the mean MPV of them is 10.845 ±0.3857 fl.

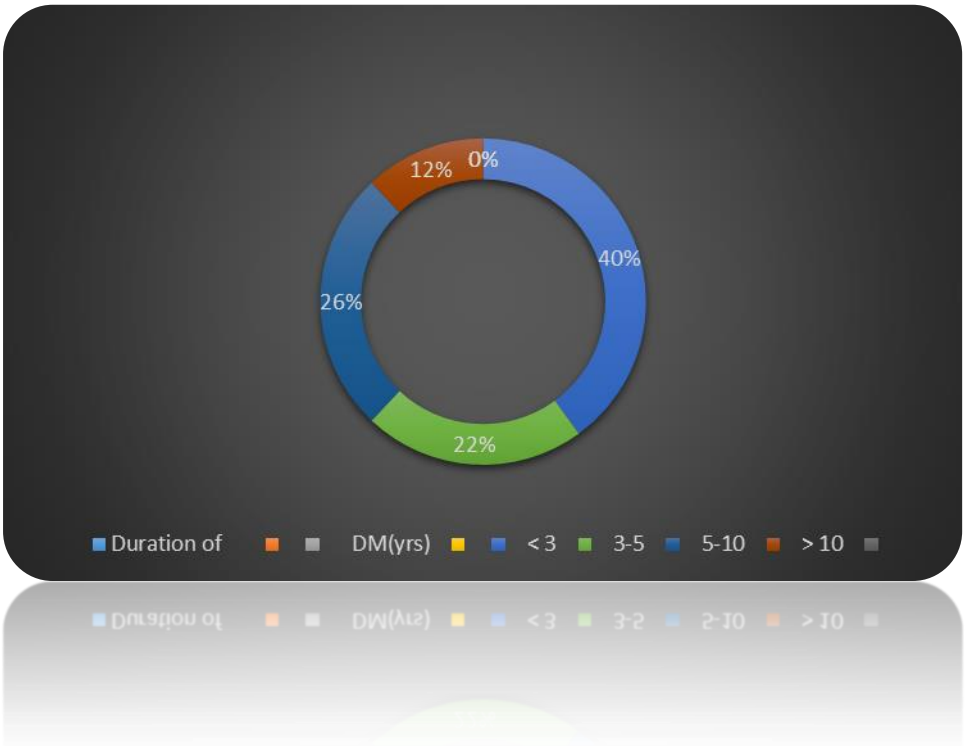
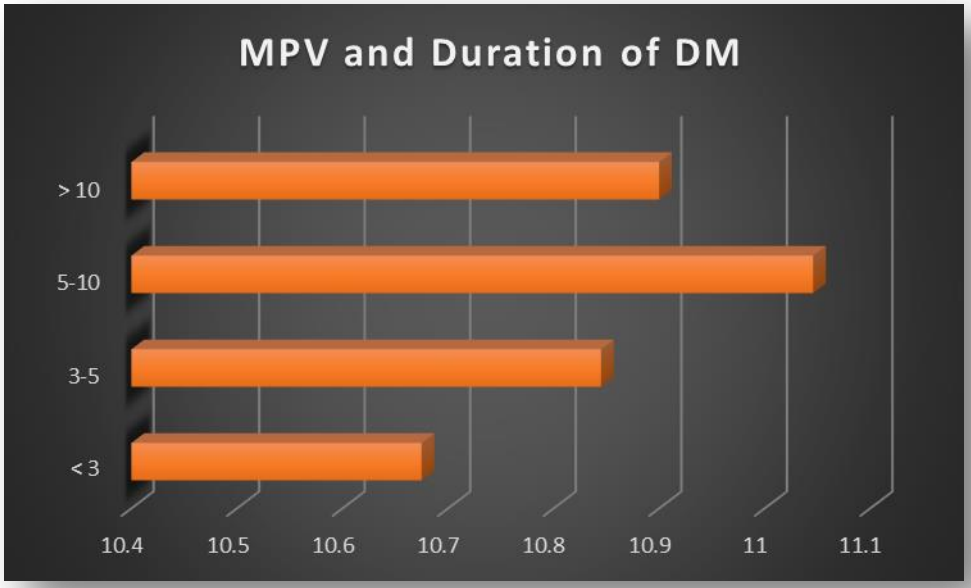


Figure 13 distribution of study population basd on Duration of diabetes

- 13 persons fall under the group with DM for 5- 10 years and the mean MPV in this category is 11.046 ±0.5238 fl.

- 6 out of the 50 in the study population had diabetes for more than 10 years and the mean MPV of this group was 10.9 ± 0.4858 fl.

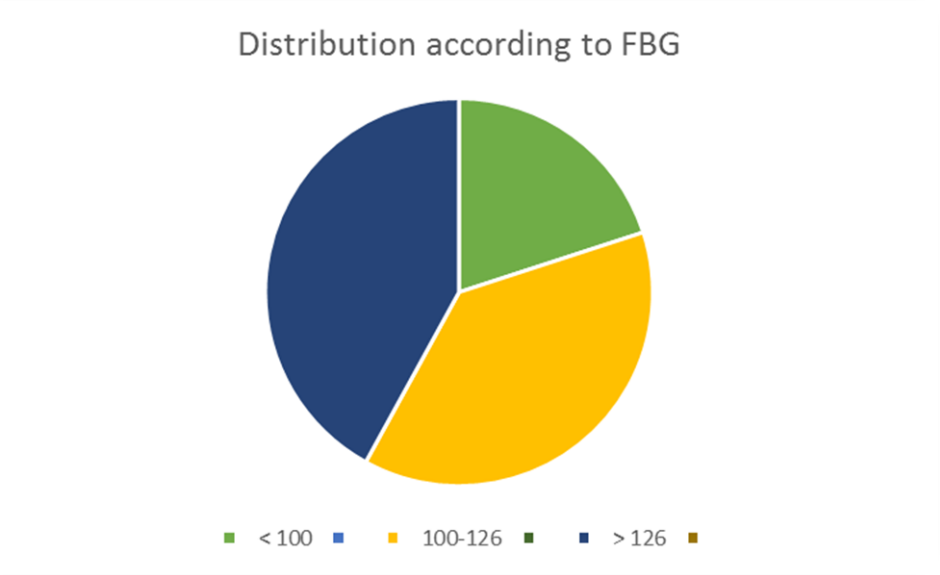
There was no statistical correlation between Mean Platelet Volume and the duration of diabetes mellitus.(p value = 0.180)



DISTRIBUTION OF STUDY POPULATION ACCORDING TO FASTING BLOOD GLUCOSE AND ITS CORRELATION WITH MPV.

Fasting blood glucose	N	Mean MPV	Std. Deviation	P value
< 100	10	10.400	.3367	.ooo **
100-126	19	10.784	.4902	
> 126	21	11.090	.3562	

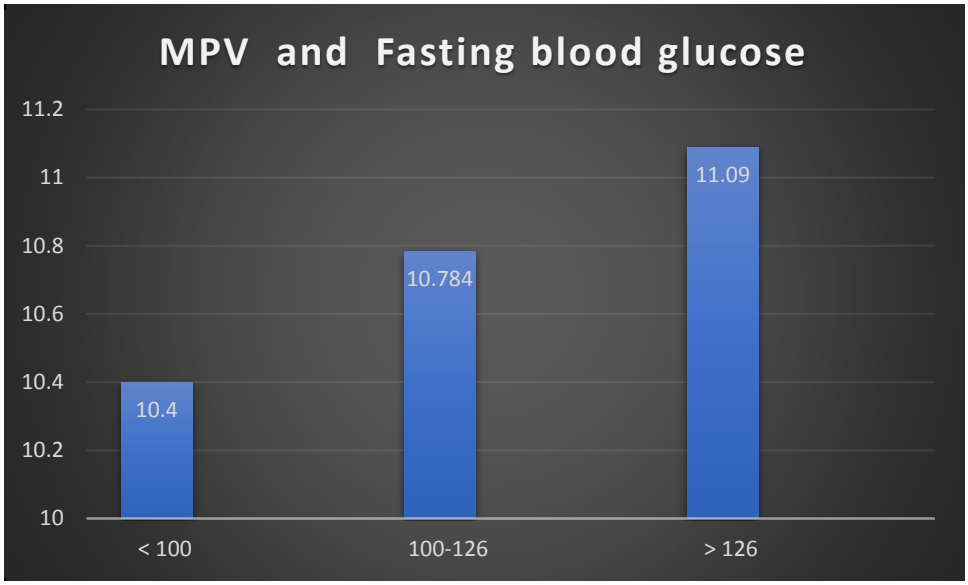
If the P value is 0.000 to 0.010 then denoted by ** it imply Significant at 1 level (Highly Significant)



- 10 among the 50 in the study population had a Fasting Blood Glucose <100 mg/dl. And the mean MPV of this group was 10.4 ± 0.3367 fl.

- 19 of them had a Fasting Blood Glucose in the range of 100-126 mg/dl and the mean MPV was 10.784 ± 0.4902 fl.

- 21 persons in the study population had a Fasting Blood Glucose >126 mg /dl and the mean MPV was 11.09 ± 0.3562 fl.



The Fasting Blood Glucose values showed a positive correlation with MPV which was statistically significant. (p value =0.000).

DISTRIBUTION OF THE STUDY POPULATION ACCORDING TO POSTPRANDIAL BLOOD GLUCOSE AND ITS CORRELATION WITH MPV.

- Among the 50 members included in the study only 1 had a post prandial blood glucose <140 mg /dl and the MPV was 9.9 fl.
- 25 members had postprandial blood glucose ranging from 140-200 mg/dl and the mean MPV of this group was 10.668 ±0.4811 fl.

- The remaining 24 persons had postprandial blood glucose values >200 mg /dl and the mean MPV of this group was 11.05 ±3587 fl.

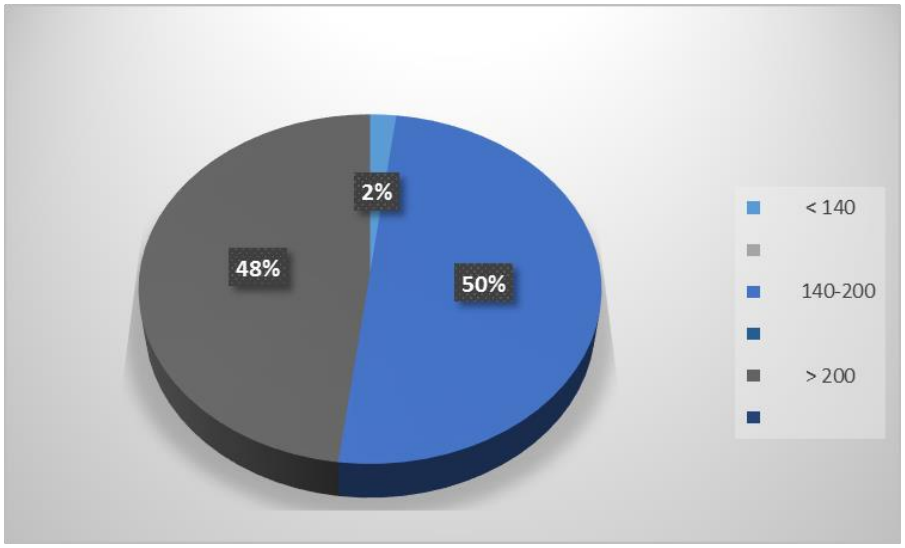


Figure 14 distribution of study population according to PPBG

Postprandial blood glucose(mg/dl)	NUMBER	Mean MPV	Std. Deviation	P value
< 140	1	9.900	.	.002*
140-200	25	10.668	.4811	
> 200	24	11.050	.3587	

- If the P value is 0.011 to 0.050 then denoted by * it imply Significant at 5 level (Significant)

Analysis of data revealed a positive correlation between postprandial blood glucose and mean platelet volume with a p value =0.002.

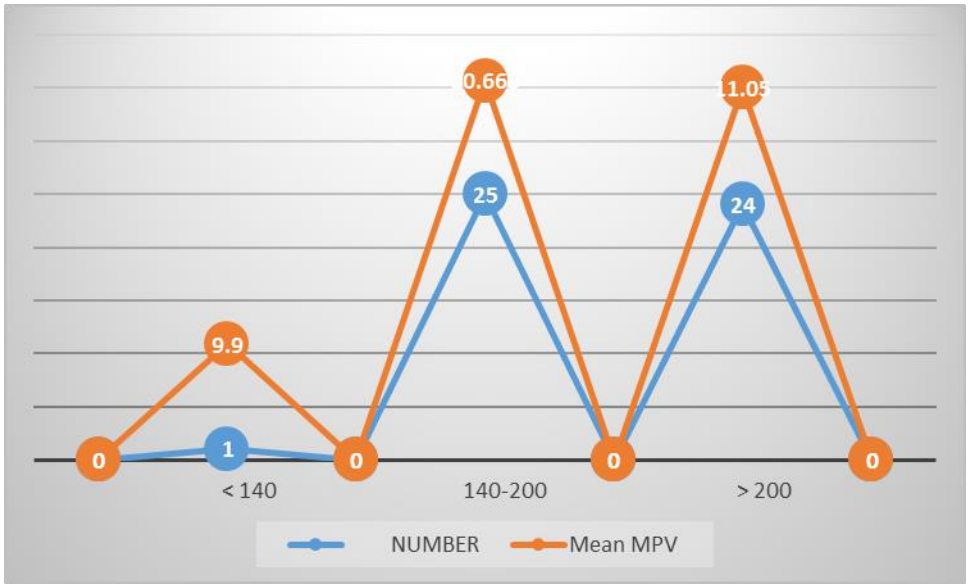
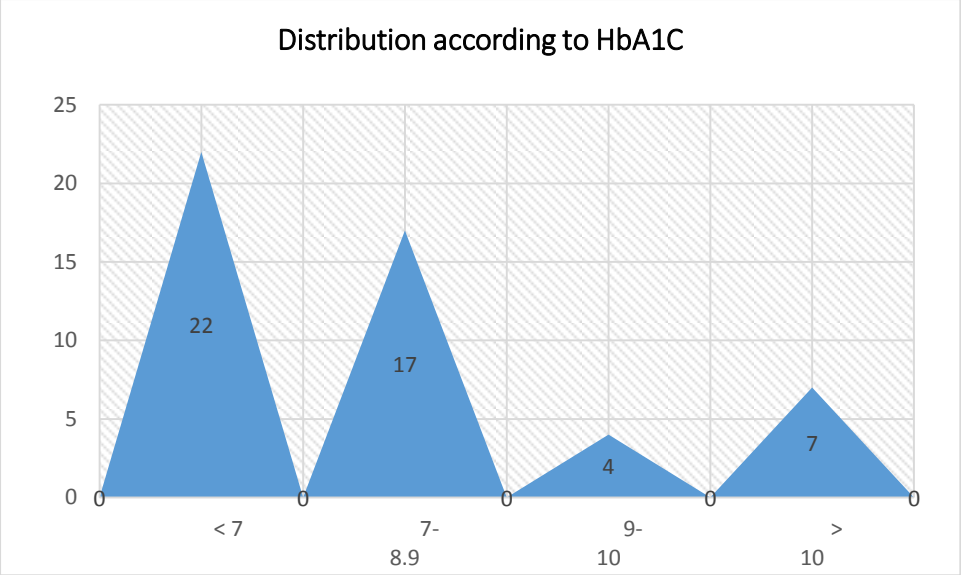


Figure 15MPV and postprandial blood glucose

DISTRIBUTION OF STUDY POPULATION ACCORDING TO HbA1C LEVELS AND ITS CORRELATION WITH MPV

- The mean HbA1C values of the study population was 7.762 .



- 22 members of the study had HbA1C <7 and the mean MPV of this group was 10.518 ± 0.3431 fl.
- 17 were categorised under the group with HbA1C 7 - 8.9 and men MPV of this class was 10.947 fl with a standard deviation of 0.4346 .

- 4 members had HbA1C values between 9 and 10 ,with the mean MPV of this group being 11.25 ±0.4123 fl.
- And 7 subjects had HbA1C >10 .the mean MPV of this group was 11.329fl with a standard deviation of 0.2498.

HbA1C	N	Mean MPV	Std. Deviation	P value
< 7	22	10.518	.3431	
7-8.9	17	10.947	.4346	.000**
9-10	4	11.250	.4123	
> 10	7	11.329	.2498	

- If the P value is 0.000 to 0.010 then denoted by ** it imply Significant at 1 level (Highly Significant)

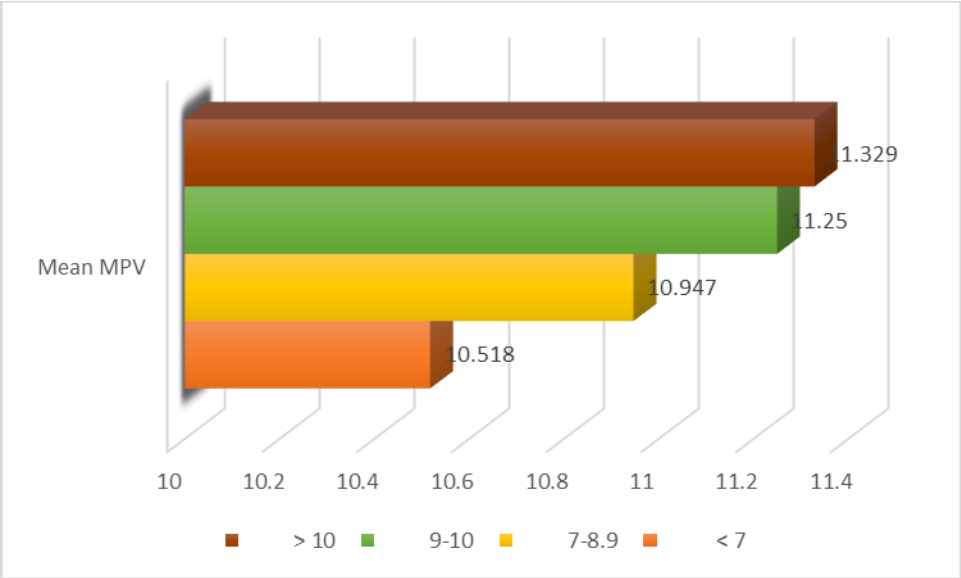


Figure 16 distribution of study population according to HbA1C

Data analysis demonstrated a positive correlation between HbA1C and

mean platelet volume. (p value = 0.000)

CORRELATION BETWEEN BETWEEN BLOOD UREA VALUES AND MEAN PLATELET VOLUME

Blood Urea	N	MPV	Std. Deviation	P value
Normal (<40 mg/dl)	45	10.818	.4638	.424
Abnormal(>40 mg/dl)	5	11.000	.6285	

Among the 50 subjects under study, blood urea was found to be elevated in 5 individuals and the average mean platelet volume of them was 11.0 fl with a standard deviation of ± 0.6285. And the urea values were normal (< 40mg/dl) in the remaining 45 subjects with mean platelet volume being 10.818 fl with a standard deviation of ±0.4638.

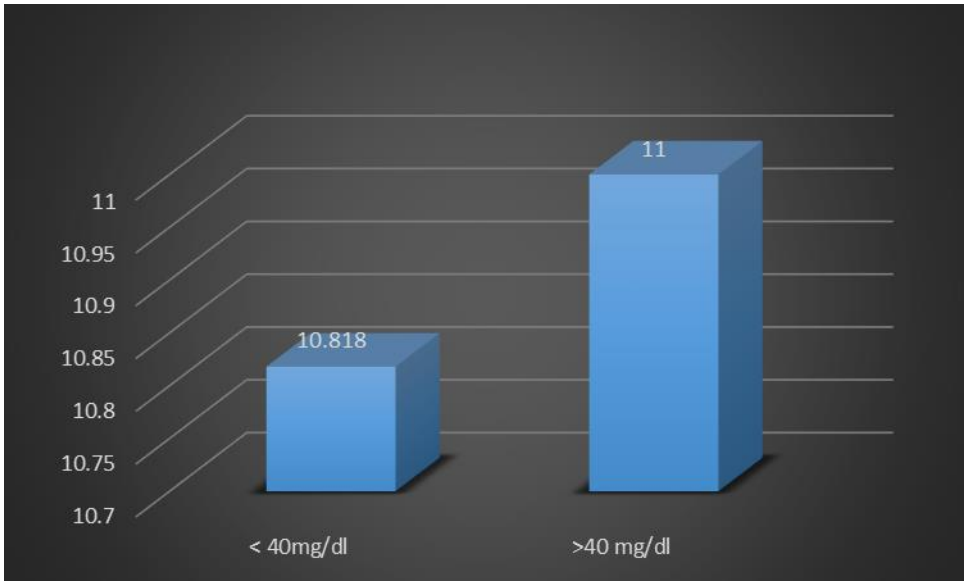


Figure 17 distribution of study population according to blood urea levels

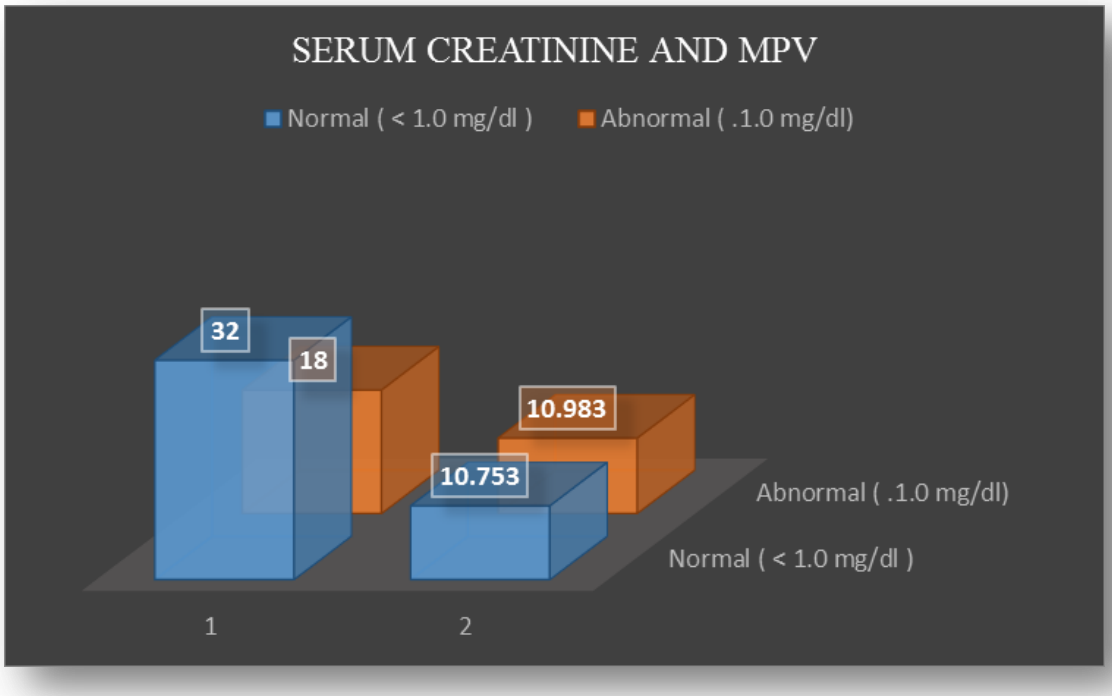
Although the MPV in subjects with an abnormal blood urea levels were higher there was no statistical significance associated with it.

CORRELATION BETWEEN SERUM CREATININE AND MEAN PLATELET VOLUME

Sr.Creatinine	N	Mean	Std. Deviation	P value
Normal (< 1.0 mg/dl)	32	10.753	.4725	0.103
Abnormal (.1.0 mg/dl)	18	10.983	.4643	

In the study population 32 people had serum creatinine values within the normal range (< 1.0 mg/dl) and the average mean platelet volume of these subjects was 10.753fl with a standard deviation of ± 0.4725 .

And the remaining 18 people had an elevated serum creatinine levels (>1.0 mg/dl) and their average mean platelet volume was 10.983 fl with a standard deviation of ± 0.4643.



The correlation between MPV and serum creatinine levels was not statistically significant . (p value = 0.103)

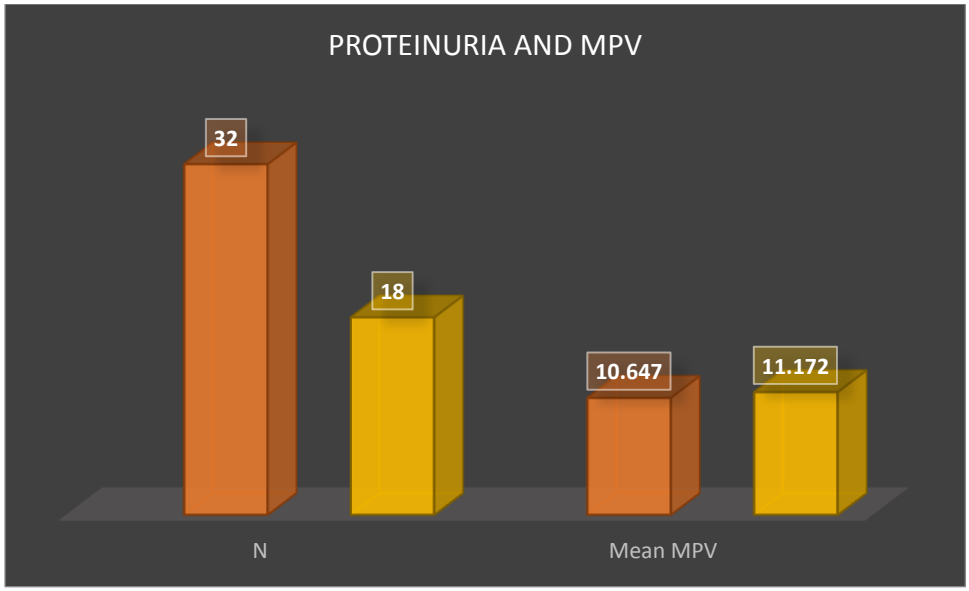
DISTRIBUTION AND CORRELATION OF PROTEINURIA AND MEAN PLATELET VOLUMES.

Among the study population of 18 members were found to have proteinuria .

proteinuria	N	Mean MPV	Std. Deviation	P value
Absent	32	10.647	.3959	.ooo**
present	18	11.172	.4309	

- If the P value is 0.000 to 0.010 then denoted by ** it imply Significant at 1 level (Highly Significant)

- the mean MPV in the group with proteinuria was found to be 11.172fl with a standard deviation of 0.4309 .
- in the group were there was no proteinuria the mean MPV was 10.647 ±3959 fl.

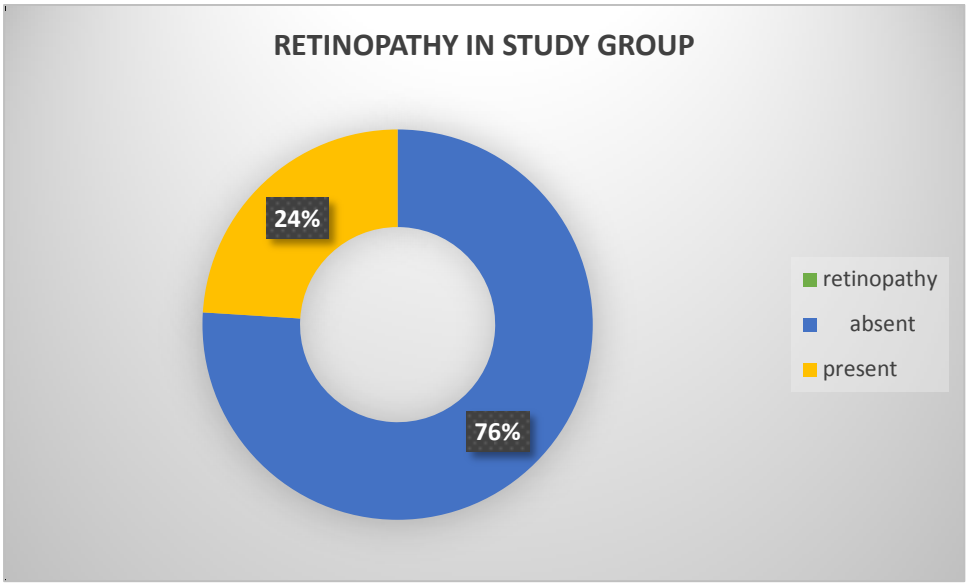


There was a positive correlation between MPV and proteinuria , MPV was significantly high in the group with proteinuria. (p value = 0.000)

CORRELATION BETWEEN RETINOPATHY AND MEAN PLATELET VOLUME

- Among the 50 subjects 12 of them were found to have various stages of diabetic retinopathy.

- And the mean platelet volume values of the group with retinopathy was higher than that without retinopathy.



- In the 12 persons with retinopathy the mean MPV was found to be 11.208 fl with standard deviation of 0.3315 .
- And the mean MPV in the non retinopathy group was 10.718 ± 0.4591 fl.

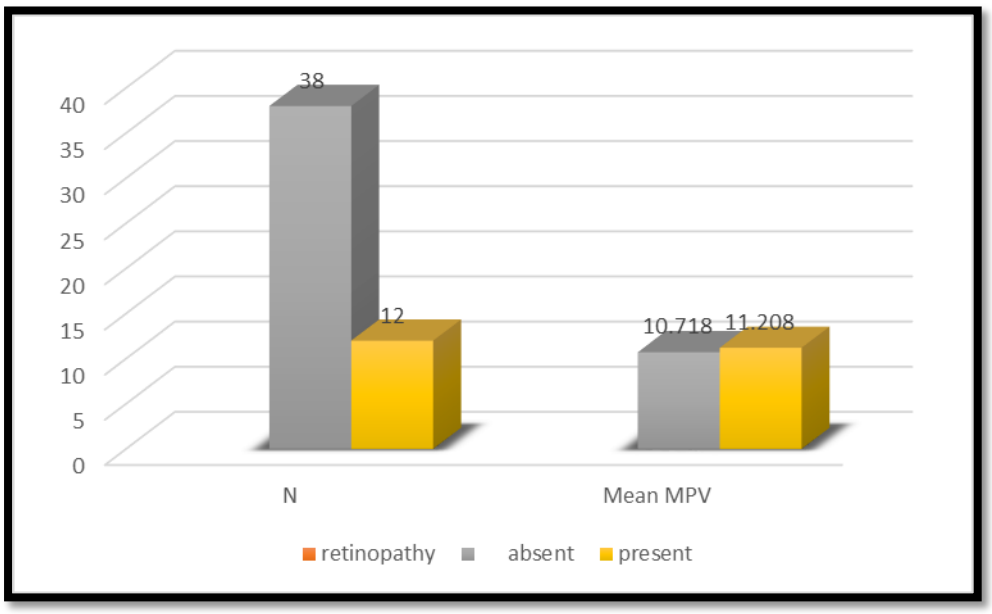
There was a positive correlation between mean platelet volume and diabetic retinopathy which was statistically significant. (p value = 0.001)

retinopathy	N	Mean MPV	Std. Deviation	P value
absent	38	10.718	.4591	.001

present	12	11.208	.3315	

- If the P value is 0.000 to 0.010 then denoted by ** it imply Significant at 1 level (Highly Significant)

Table 3 MPV and retnopathy correlation



COMPARISON OF MEAN PLATELET VOLUME IN TYPE 2 DIABETICS TAKING INSULIN AND ORAL HYPOGLCAEMIC AGENTS.

- In the study population of 50 , 19 members were found being treated with insulin and the remaining 31 of them were on oral hypoglycaemic agents.

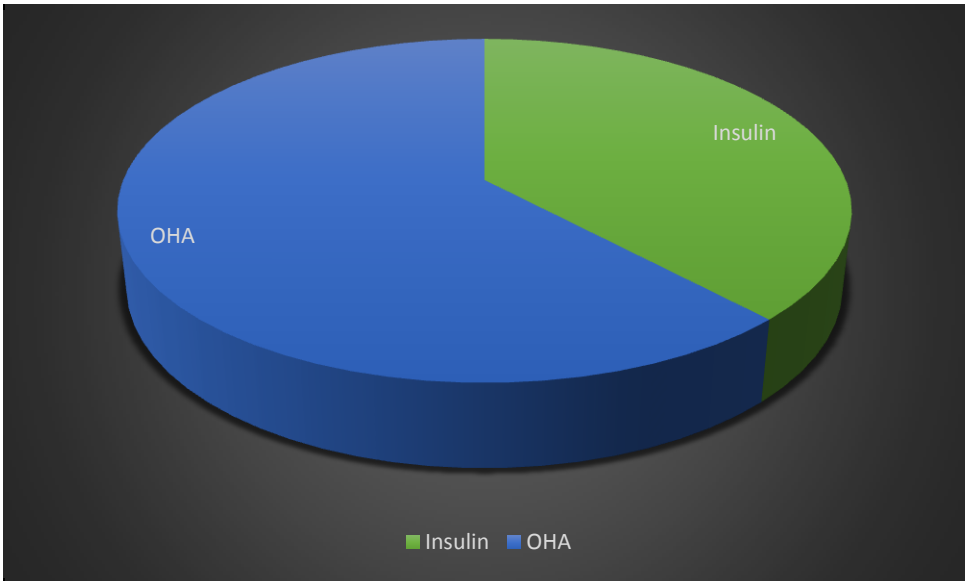


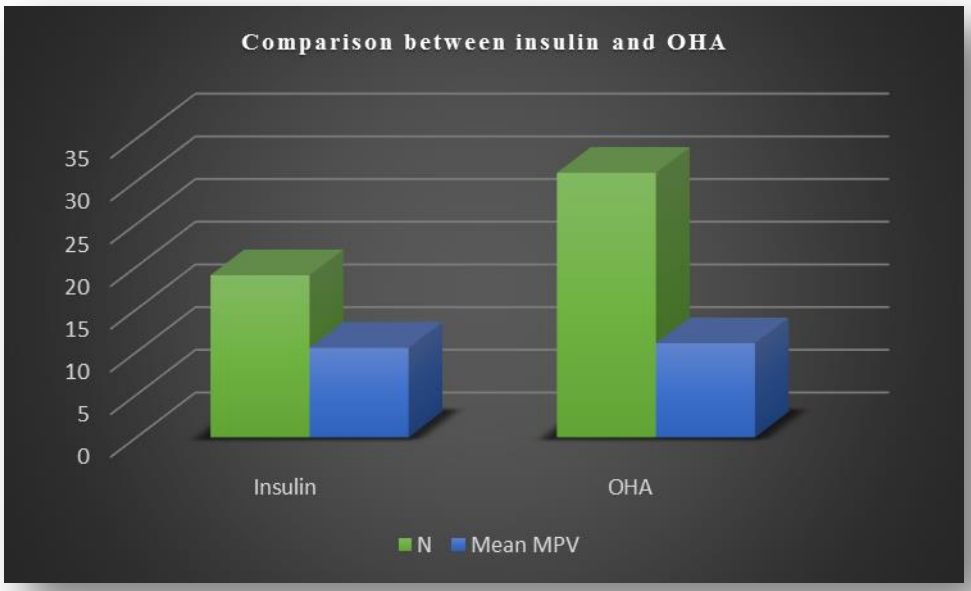
Figure 18 distribution of study population based on therapy

- The group with insulin was found to have a mean MPV ehich was much lower than the OHA group.
- The mean MPV of the insulin group was 10.505 fl with astandard deviation of 0.3535 .
- The mean MPV in the group taking oral hypoglcaemic agents was found to be 11.039 fl with a standard deviation of 0.4318.

Treatment modality	N	Mean MPV	Std. Deviation	P value
Insulin	19	10.505	.3535	<0.001**
OHA	31	11.039	.4318	

- If the P value is 0.000 to 0.010 then denoted by ** it imply Significant at 1 level (Highly Significant)

There was a definite correlation between MPV and both the groups. The MPV of the subjects on insulin therapy was lower than the OHA group. And this difference in mean platelet volume between the 2 groups was found to be statistically significant . (p value <0.001



Thus at the end of the study age, duration of diabetes mellitus , body mass index, blood urea levels, serum creatinine values did not have any statistically significant correlation with mean platelet volume.

And the levels of **fasting blood glucose, postprandial blood glucose , HbA1C, proteinuria and retinopathy** was found to have a positive correlation with mean platelet volume.

DISCUSSION

The mean MPV value of the study population of 50 diabetic individuals was 10.836 fl and this is higher than that of the normal healthy population. This is in accordance to the study published by Kodiatte et al,, Hekimsoy et al., Demirtunc et al., Zuberi et al., Atea et al., Jindal et al., and Papanas et al. where the mean MPV was 8.27 ±0.74 fl. The study included 24 females and 26 males and the mean age of the study population was 52.56 years , and the study by Pradeep et al had a mean age of 56.9 years.and the mean duration of diabetes among the study population was found to be 11.15 years and the study by Kodiatte et al had a mean duration of diabete at 6.2 years.

A study by Shah B et al demonstrated a significant association between mean platelet volume and the presence and severity of diabetes.and this association was more evident in the group with poor glycaemic control.

The body mass index (fir the Asian population) on this study did not have any corre;ation with MPV ,this again is in accordance to the study by Coban et al who shoed that there was no correlation between the two. The mean BMI of the study population was 25.97 kg/m2.

The mean fasting blood glucose values of the study population was 131.42 ± 45.4 mg/dl. In the study by Kodiatte et al the mean fasting blood glucose value was 151.5 ± 71.7 mg/dl. There was positive correlation between

the fasting blood sugar values and mean plaetelet volume.this outcome is reiterated by the study results of Kodiatte et al also having similar outcome.

The postprandial blood glucose values in the study by Kodiatte et al had a mean of 252.9 ±94.85 mg/dl. While in our study group the mean PPBS values were 219.72 ±74.6 mg/dl.and there was a positive correlation between PPBS values and mean platelet volume similar to that in the former.

The HbA1C values in our study population was 7.762 ± 1.83
While that of the study by Kodiatte et al was 9.13 ±2.53 . our study revealed a positive correlation between HbA1C levels and mean platelet volume, this is in accordance to the former publication of Kodiatte et al.

The mean platelet volume values of subjects in the study population was not found to correlate with the blood urea and serum creatinine values.

The values of mean platelet volume was higher in diabetics with proteinuria than in those who lacked it.And there was a significant positive correlation between proteinuria and MPV .thus indicating a correlation existing between MPV and diabetic nephropathy.

Likewise the MPV values were higher in subjects with diabetic retinopathy and there was a definite positive correlation between the two.

In our study the MPV was significantly high in patients on oral hypoglycaemic agents rather than those on insulin therapy. The mean MPV of the group with insulin therapy was 10.505 ±0.3535fl and the mean MPV in the OHA group was 11.039 ± 0.4318 fl. This derivation was statistically significant (p value = 0.000), and it is in accordance to the outcome of the study by Pradeep.V et al. This indicates that a strict glycaemic control with early initiation of insulin can reduce platelet hyperactivity.

In our study the MPV levels were higher in subjects with microvascular complications , and is supported by other studies like Ates et al. [12] and Papanas et al and Kodiatte et al.This indicates that diabetes mellitus has a significant influence on the platelet function and microvascular complications have a definite association with platelet hyperactivity.

LIMITATIONS OF THE STUDY

This study has considered a population of 50 and thus the appropriate representation of the population and better outcomes could be attained by increasing the sample size. The restriction of study to small geographical area is also a constraint.

CONCLUSION

- Mean platelet volume values were significantly lower in diabetic people on insulin therapy than those on oral hypoglycaemic agents.

- There was a positive correlation between mean platelet volume and microvascular complication indicating a higher platelet activity in this subgroup.

- Emphasis should be laid on a strict glycemic control and early initiation of insulin therapy in order to prevent the vascular complications associated with diabetes.

- Mean platelet volume can serve as a cost effective marker of atherothrombosis and helps monitor platelet activity.

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name	age	ht	wt	BMI	duration of DM	family	treatment	FBS	PPBS	HbA1C	Bld.urea	sr.crea	u. albu	u.su	fundus	neurop	platel	MPV
sudhakar	46	1.72	65	#####	5	Y	OHA	142	210	7.8	32	0.8	nil	nil	normal	nil	2.89	10.6
muneswari	67	1.54	67	#####	14	N	OHA	270	347	11.5	40	1.1	2#	1#	mod npdr	P	2.67	11.5
kasthuri	60	1.56	58	#####	7	N	OHA	111	170	7.2	28	0.9	nil	nil	normal	nil	3.12	11
syed musta	53	1.62	74	#####	10	Y	insulin	116	178	6.9	24.2	1.3	2#	nil	pdr,photoc	P	3.25	11.2
vijaya	46	1.58	68	#####	7	Y	OHA	276	318	11.6	29.6	1.1	nil	nil	normal	nil	2.89	11.5
sekar	60	1.74	78	#####	1	N	OHA	87	130	6.5	18.8	0.8	nil	nil	normal	nil	3.3	9.9
kantha	65	1.62	70	#####	15	N	OHA	145	278	8.4	34.2	1.6	2#	nil	mod npdr	P	3.67	11.2
kadumbadi	62	1.5	80	#####	4	N	OHA	118	182	7.6	30	1.1	nil	nil	normal	nil	2.67	10.6
thahira beg	62	1.45	62	#####	20	Y	insulin	95	194	6.4	38.3	1.8	3#	2#	severe npc	P	2.41	10.7
seeni ibrah	44	1.75	76	#####	1.5	N	OHA	151	301	10.6	28.4	1	nil	nil	normal	nil	2.78	10.9
akthar basl	44	1.6	57	#####	11	N	OHA	110	247	7.4	26	1	micro	nil	normal	nil	3.1	11.1
janarthanar	66	1.72	72	#####	5	Y	insulin	105	177	6.8	35	0.9	nil	nil	normal	P	3.65	10.4
kathirunish	29	1.5	49	#####	4	Y	OHA	108	301	9.2	24.8	0.8	nil	nil	normal	nil	2.45	10.8
selvam	41	1.56	59	#####	3	Y	insulin	100	184	6.6	16.9	0.9	nil	nil	normal	nil	3.09	9.9
muthukuma	58	1.64	70	#####	10	N	insulin	96	159	5.9	36	1	nil	nil	normal	nil	2.85	10.2
sheila shal	46	1.58	68	#####	5	Y	OHA	142	199	7.1	29	1	nil	nil	normal	nil	3.9	11
renganatha	72	1.7	72	#####	14	N	insulin	111	169	6.8	36	1.1	microa	nil	normal	nil	2.49	10.8
mariappan	46	1.68	74	#####	3	N	OHA	98	154	6.5	26	0.9	nil	nil	normal	nil	2.98	10.7
robert	42	1.74	76	#####	2	Y	insulin	125	186	7.4	35	1.1	nil	nil	normal	nil	3.15	10.2
jency	56	1.55	64	#####	7	N	OHA	174	258	9	41	1.2	1#	nil	mild npdr	P	2.94	11.6
kavitha	52	1.5	63	#####	6	N	OHA	102	178	7.8	38	1	microa	nil	normal	nil	3.45	12.1
usman	64	1.6	60	#####	9	N	insulin	94	176	6.5	38.4	0.9	nil	nil	normal	nil	2.78	10.6
angaiyakar	61	1.52	62	#####	6	N	insulin	122	211	7	26	0.87	nil	nil	normal	nil	2.83	10.5
dinesh	45	1.68	65	#####	1	Y	OHA	104	220	7.1	18.9	0.97	nil	nil	normal	nil	2.67	10.9
selvakuma	54	1.64	64	#####	2	Y	OHA	88	165	6.6	26.8	1	nil	nil	normal	nil	3.18	10.7
sampath	50	1.75	76	#####	3	N	insulin	114	192	6.5	26	1	nil	nil	normal	nil	2.89	9.8
harini	47	1.52	62	#####	2	N	OHA	143	304	10.3	31.6	0.88	2#	nil	mod npdr	P	3.26	11.6
uma mahes	43	1.5	68	#####	2	Y	insulin	111	195	7.1	33	1.1	nil	nil	normal	nil	2.52	10.4
siddarth	39	1.64	73	#####	1	Y	OHA	112	195	7	29.2	0.9	nil	nil	normal	nil	2.99	10.8
menaka	53	1.64	68	#####	5	N	OHA	143	277	8.6	38	1.2	1#	nil	normal	nil	2.79	11.1
nirmala	44	1.56	74	#####	3	Y	insulin	204	357	9.1	40	1	microa	nil	normal	nil	3.22	11
amaiappan	68	1.66	70	#####	12	N	insulin	99	174	6.2	42	1.2	1#	nil	normal	P	2.9	10.1
kaniappan	61	1.56	61	#####	10	N	OHA	242	287	10.7	52	1.5	3#	1#	mild npdr	P	3.35	11.4
kanmani	57	1.52	63	#####	5	N	insulin	132	224	6.8	26.4	0.89	nil	nil	normal	nil	2.64	10.7
muthumeer	48	1.6	66	#####	3	N	OHA	127	211	6.9	33	0.85	nil	nil	normal	nil	2.73	10.7
sulaiman	53	1.73	83	#####	8	Y	OHA	137	253	7.9	36	1.3	1#	nil	mod npdr	P	2.87	11.2
arumugam	55	1.66	80	#####	6	Y	insulin	130	201	6.9	40.3	1.1	nil	nil	mild npdr	P	2.56	10.6

aathilakshr	57	1.54	73	#####	9	Y	insulin	120	185	6.6	33	0.9	nil	nil	normal	nil	2.76	10.8
ranjith	46	1.6	65	#####	3	Y	OHA	95	155	6.5	25.7	1	nil	nil	normal	nil	2.68	10.6
latha	49	1.54	59	#####	3	N	OHA	112	198	7.5	30.4	0.96	nil	nil	normal	nil	3.07	11
subramania	64	1.58	63	#####	5	N	insulin	103	178	6.5	22.9	0.89	nil	nil	normal	nil	3.12	10.5
bazeera	44	1.48	57	#####	3	N	OHA	130	214	7.2	34	1	1#	nil	normal	nil	2.68	10.9
jothi	45	1.55	58	#####	2	N	insulin	107	195	6.7	38	1	nil	nil	normal	nil	3.33	10.6
kannan	56	1.64	65	#####	7	N	OHA	133	256	7.4	26.7	1.1	1#	nil	mild npdr	P	3.45	10.9
shanmugar	60	1.6	61	#####	5	N	insulin	97	188	6.8	36	0.83	nil	nil	normal	nil	2.67	10.6
bhavani	55	1.6	57	#####	2	Y	OHA	153	218	7.4	27	1	nil	nil	normal	nil	2.82	10.5
neelakanda	46	1.68	74	#####	3	Y	OHA	241	375	11.6	40.6	2.1	3#	1#	severe npc	P	2.56	11.3
chithra	44	1.48	58	#####	1	N	OHA	143	235	10.5	33.6	1	nil	nil	mild npdr	nil	2.92	11.1
sudha	47	1.52	60	#####	4	N	OHA	135	244	9.6	38.5	0.99	nil	nil	normal	nil	2.64	11.6
manikanda	56	1.7	65	#####	5	Y	OHA	118	183	7.6	37.9	1.2	2#	nil	mod npdr	P	2.93	11.4

INSTITUTIONAL ETHICAL COMMITTEE
GOVT.KILPAUK MEDICAL COLLEGE,
CHENNAI-10
Ref.No.2212/ME-1/Ethics/2014 Dt:03.04.2014.
CERTIFICATE OF APPROVAL

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval "A Study on Comparison of mean platelet volume in type 2 diabetics on insulin tehrapy an oral hypoglycaemic agents " – For Project Work submitted by Dr.Rukmani Prabha, MD (GM), PG Student, KMC, Chennai-10.

The Proposal is APPROVED.

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

GOVT. KILPAUK MEDICAL COLLEGE
30 MAY 2014
Kilpauk, Chennai-10.

30/5/14.

CHAIRMAN,
Ethical Committee
Govt.Kilpauk Medical College,Chennai

ABBREVIATIONS

MPV= MEAN PLATELET VOLUME

BMI= BODY MASS INDEX

FFA= FREE FATTY ACID

HDL= HIGH DENSITY LIPOPROTEIN

LDL= LOW DENSITY LIPOPROTEIN

DM= DIABETES MELLITUS

HLA= HUAMN LEUKOCYTE ANTIGEN

IR= INSULIN RESISTANCE

OGTT= ORAL GLUCOSE TOLERANCE TEST

GLUT= GLUCOSE TRANSPORTER

IRS= INSULIN RECEPTOR SUBSTRATE

CAD= CORONARY ARTERY DISEASE

CPT= CARNITINE PALMITOYL TRANSFERASE

PPAR= PEROXISOME PROLIFERATOR ACTIVATOR RECEPTOR

TNF= TUMOUR NECROSIS FACTOR

PKC= PHOSPHOKINASE C

TGF= TRANSFORMING GROWTH FACTOR

CRP= C REACTIVE PROTEIN

IL= INTERLEUKIN

VLDL= VERY LOW DENSITY LIPOPROTEIN

TIA= TRNASIENT ISCHAEMIC ATTACK

TLR= TOLL LIKE RECEPTOR

NCP= NON COLLAGEN PROTEIN

CAM= CELL ADHESION MOLECULES

PROFORMA

NAME : OCCUPATION:

AGE/SEX: ADDRESS:

Duration of DM:

Treatment hist and duration:

Compliance:

Hist relating to complications of DM:

Past hist and drug intake:

Personal hist:

PHYSICAL EXAMINATION:

Height Weight

BMI:

Vitals :

CVS :

RS :

Neurological examination:

Fundus examination:

INVESTIGATIONS:

1.FBG

2. 2 hr PPBG

3.HbA 1C

4. CBC

5. MPV

6.LIPID PROFILE

7. BLOOD UREA

8.SERUM CREATININE

9.URINE ALBUMIN

10.URINE SUGAR

11. ECG

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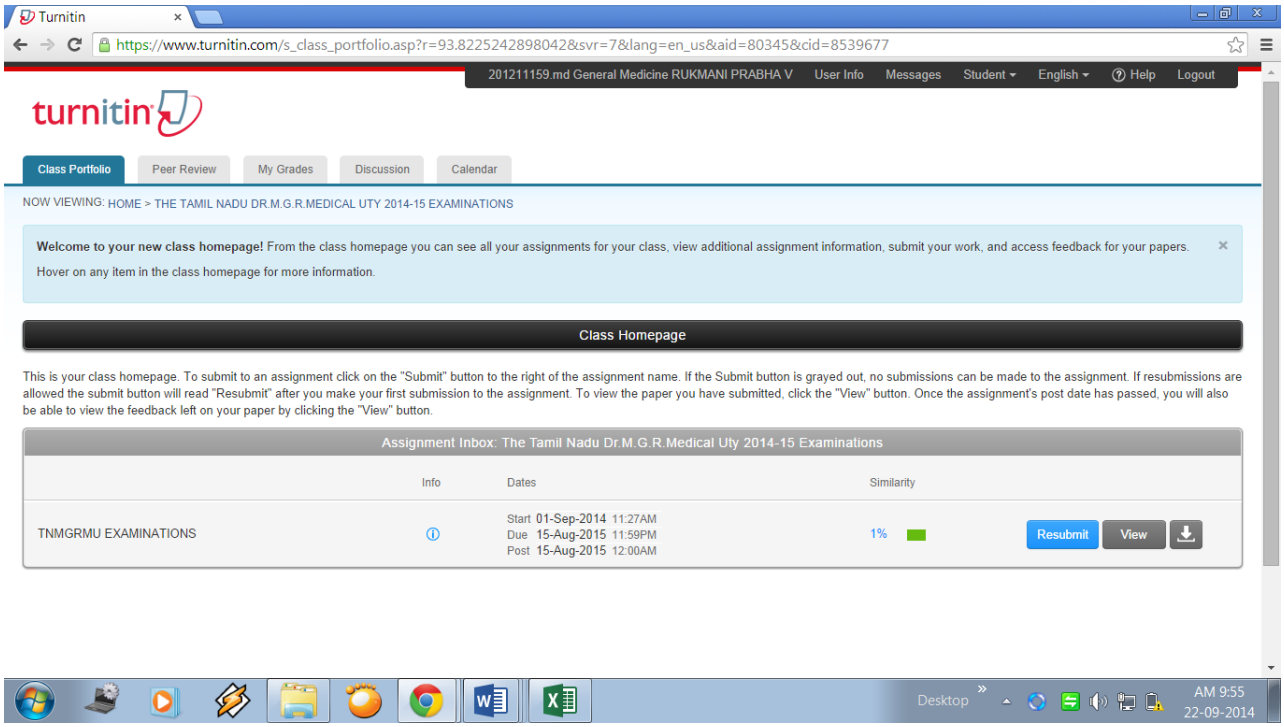

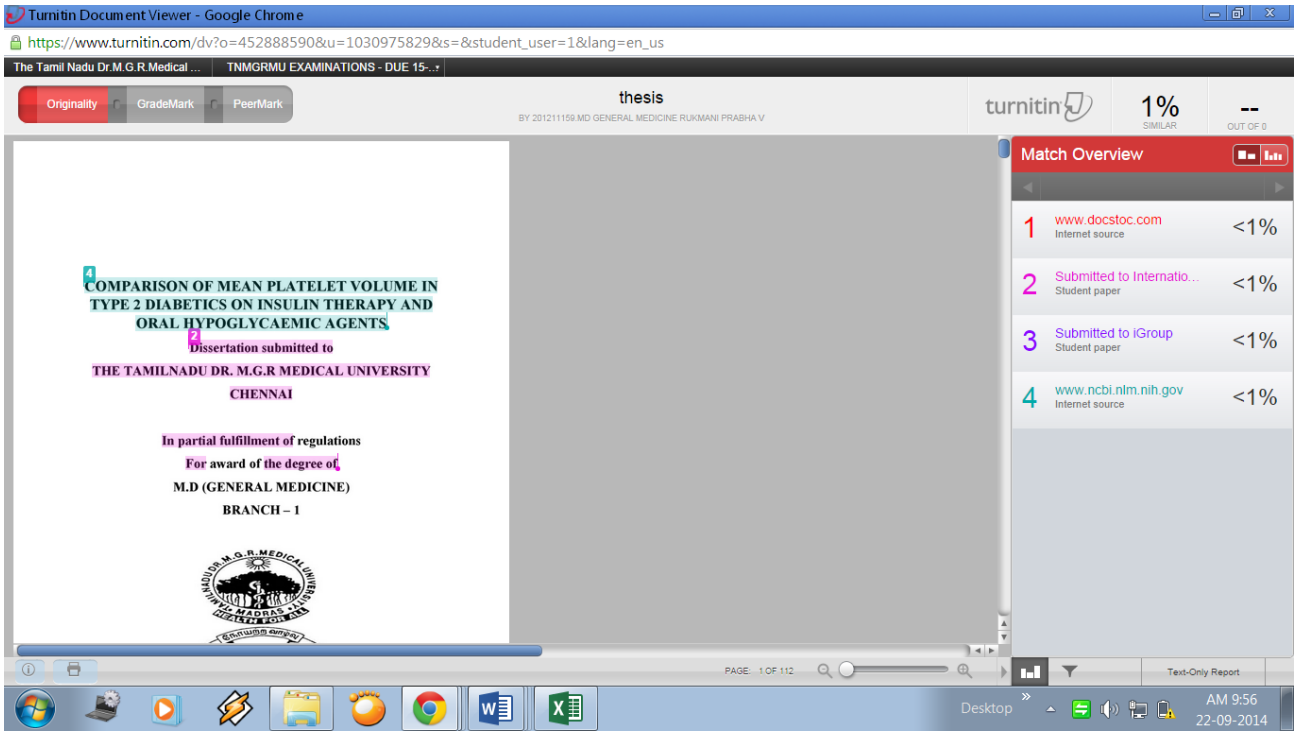
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Dissertation submitted to
THE TAMILNADU DR. M.G.R MEDICAL UNIVERSITY
CHENNAI
In partial fulfillment of regulations
For award of the degree of
M.D (GENERAL MEDICINE)
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